PHARMACOKINETICS AND PHARMACODYNAMICS OF CORTICOSTEROID PRODRUGS AND SOFT DRUGS

By

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This work is dedicated to my parents, my husband, and my son.

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Requirements for the Degree of Doctor of Philosophy

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By

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December 2004

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The overall objective of this work is to show that the administration of corticosteroid prodrugs and soft drugs facilitates the use of corticosteroids for both local and systemic administration. The first part of this study evaluated the pharmacokinetics of the prodrug methylprednisolone hemisuccinate in patients with acute spinal cord injury. Concentrations of the active metabolite methylprednisolone in plasma and cerebrospinal fluid (CSF) were sufficiently high to induce non-receptor mediated unspecific membrane effects.

Next, based on linear and nonlinear protein-binding characteristics, we developed an application to predict the dose-dependent pharmacokinetics of prednisolone after intravenous administration of the prodrug prednisolone sodium phosphate and after oral administration of prednisolone. We linked this application to the pharmacodynamic effect using an indirect response model. We obtained plasma concentration-time data of free and total prednisolone from previous studies, and then determined the pharmacokinetic

(PK) parameters by curve fitting. Four MS Excel spreadsheets were designed to simulate concentration-time profiles and the systemic effects for a new drug showing nonlinear protein binding. Furthermore, they allow comparison of the results with prednisolone, one of the most commonly prescribed corticosteroids, to assess the effect on cumulative cortisol suppression (which, in turn, is related to the side effects profile). These spreadsheets increase our understanding of how nonlinear protein binding affects the pharmacokinetics and pharmacodynamics of prednisolone, as well as other exogenous corticosteroids.

Finally, we investigated the receptor binding affinities to the rat glucocorticoid receptor in the lung; the pharmacokinetics after IV administration to rats; and the protein binding to human plasma proteins for the soft steroids loteprednol etabonate, etiprednol dicloacetate, and some new investigational compounds (929, D45, 904, 453, and 423). All of the test compounds (except Compound 423) showed receptor binding affinities similar to or exceeding those of the reference dexamethasone. This was in general agreement with their chemical structure. The protein binding was high, with fractions bound consistently over 96%. The pharmacokinetics of Compound 929 were further investigated in detail (by noncompartmental and compartmental data analysis), and showed a rapid disappearance of the drug after IV administration.

CHAPTER 1
INTRODUCTION

Corticosteroids are some of the most important anti-inflammatory and immunosuppressive agents in the pharmacological treatment of several diseases including asthma, allergic rhinitis, rheumatoid arthritis, inflammatory bowel diseases, rejection of organ transplants, and shock symptoms. The ability of corticosteroids to act on different target tissues and exert biological responses depends in most cases on the presence of the glucocorticoid receptor (GR). The GR belongs to the large family of ligand-activated transcription factors, that includes receptors for the steroid hormones, thyroid hormone, retinoic acid, and vitamin D [1]. It is an intracellular receptor, located in the cytoplasm. Binding of a ligand to the receptor initiates a series of cellular events involving synthesis of new proteins [2]. Although there are two different types of GR, currently available glucocorticoids predominantly bind to the type II receptor. Because of the ubiquitous nature of the GC receptor, corticosteroids act on a wide variety of cell types, which accounts for their many therapeutic indications, and also accounts for their negative side effects such as lymphopenia, hyperglycemia, osteoporosis, changes in bone-mineral density, growth retardation in children, cataracts, glaucoma, skin thinning, and suppression of the hypothalamus-pituitary-adrenal (HPA) axis due to a negative feedback mechanism [3]. Furthermore, corticosteroids in high doses also show non-receptor mediated (antioxidant, membrane-stabilizing) effects.

A prodrug is a pharmacologically inactive compound. Because of changes of the physicochemical, biopharmaceutical, or pharmacokinetic properties of a prodrug, the

amount of active drug that reaches its site of action is maximized. To exert a local effect, they must be activated through enzymatic or non-enzymatic reactions in the target tissue (e.g., lung or nose).

Administering a prodrug can improve drug penetration through biological membranes, and can improve the stability and solubility of a drug. The prodrug principle for increasing solubility has been successfully implemented in the case of intravenous administration of methylprednisolone hemisuccinate, and prednisolone sodium phosphate. It is also possible to decrease the toxicity of a drug, increase the pharmacological activity, and increase its site specificity (as has been done with corticosteroids). For corticosteroids used in inhalation and intranasal therapy, activation is usually initiated by esterases in the lung/nose, which results in an increased retention time of the drug in the lung. This tissue specificity also reduces the risk of local side effects if the esterases necessary for the activation are only found in the lung tissue. In other words, desired characteristics of a prodrug are a low or no receptor binding, rapid hydrolysis to the active compound in the lung or nose, specific targeting to the lung/nose, high receptor binding affinity of the active compound, and no pharmacological effect of its secondary metabolites.

Another possibility to improve the therapeutic ratio (topical : systemic ratio) is the concept of soft drugs. A soft drug is defined as a biologically active chemical compound characterized by a predictable and controllable *in vivo* metabolism to nontoxic moieties, after it achieves its therapeutic role [4]. The inactivation occurs ideally in a single step, although further metabolism of the inactive metabolite is possible [5]. The metabolic inactivation occurs preferably through hydrolysis, without the use of enzymes that could

be saturated. Therefore, a soft drug would show high concentrations at the site of action, but would be inactivated rapidly in the liver once it reaches the systemic circulation. This would be an advantage to currently available corticosteroids, because systemic side effects could be minimized and the therapeutic ratio could be increased.

Of the many indications and synthetic corticosteroids, we focus here on the systemically administered prodrugs methylprednisolone hemisuccinate (MPHS) (Figure 1-1) for acute spinal cord injury and prednisolone sodium phosphate (PN) (Figure 1-2) for inflammatory diseases in general.

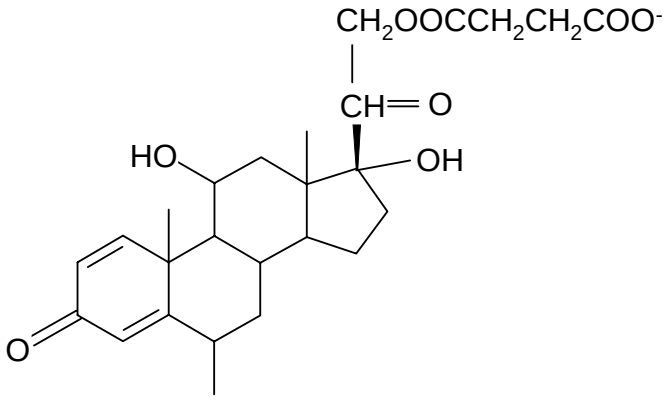


Figure 1-1. Structure of methylprednisolone hemisuccinate

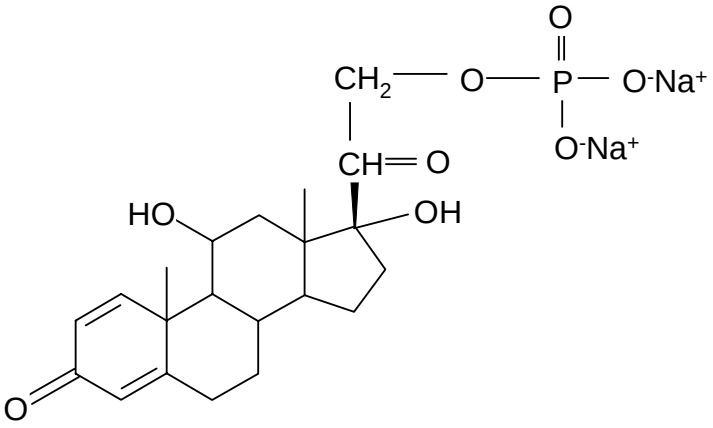
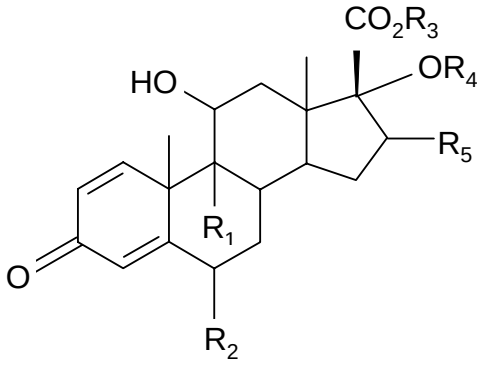


Figure 1-2. Structure of prednisolone sodium phosphate

For the soft drug approach, we chose the locally administered soft steroids loteprednol etabonate (LE) for the treatment of ocular and nasal inflammations and asthma; etiprednol dicloacetate (ED), and several new soft steroids intended for use in asthma therapy (Figure 1-3).



	R1	R2	R3	R4	R5
LE	H	H	CH_2Cl	$CO_2C_2H_5$	H
ED	H	H	CH2CH3	$COCHCl_2$	H
929	H	CH_3	CH_2Cl	$CO_2C_2H_5$	H
D45	Cl	H	CH_2Cl	$CO_2C_2H_5$	H
904	F	F	CH_3	$COCHCl_2$	CH_3
453	F	H	CH_3	$COCHCl_2$	CH_3
423	F	H	CH_3	COCH2Cl	CH_3

Figure 1-3. Structure of loteprednol etabonate, etiprednol dicloacetate and other novel soft steroids

The overall objective of this work was to show that the administration of corticosteroid prodrugs and soft drugs facilitates the use of corticosteroids for both local and systemic administration. Methylprednisolone hemisuccinate and prednisolone sodium phosphate were chosen as test compounds for the prodrug approach; loteprednol etabonate, etiprednol dicloacetate, and other novel compounds were chosen for the soft drug approach. To test the overall hypothesis the following specific aims were designed:

1. Investigate the pharmacokinetics of the prodrug methylprednisolone hemisuccinate (MPHS) and its metabolite methylprednisolone (MP) in motorcycle accident victims with acute spinal cord injury (ASCI) using Nonlinear Mixed Effect Modeling (NONMEM).

2. Develop an application to predict the dose-dependent pharmacokinetics of prednisolone after intravenous administration of prednisolone sodium phosphate and oral administration of prednisolone based on linear and nonlinear protein binding characteristics, and to link those to the pharmacodynamic effects using an indirect response model.

3. Investigate the receptor binding affinities to the rat glucocorticoid receptor in the lung, the pharmacokinetics after IV administration to rats, and the protein binding to human plasma proteins for the soft steroids loteprednol etabonate, etiprednol dicloacetate, 929, D45, 904, 453, and 423.

CHAPTER 2
LITERATURE OVERVIEW

Methylprednisolone in the Treatment of Acute Spinal Cord Injury (ASCI)

Spinal cord injury (SCI) results from an insult inflicted on the spinal cord compromising (completely or incompletely) its major functions such as motor, sensory, autonomic, and reflex [6]. It occurs all over the world, with an incidence of 15 to 40 cases per million. The cause of these injuries ranges from motor vehicle accidents, falls and community violence to sports and recreational activities and workplace-related injuries. Of these causes, the older population tends to be affected more by falls, while younger victims are more prone to injuries from motor vehicle accidents and recreational activities. Mortality from acute spinal cord injuries (ASCI) is between 48.3 and 79% either at the time of the accident or on arrival at the hospital [7]. However, although most acute spinal cord injuries are traumatic, they may also result from degenerative spine disease, ischemia, demyelination, and inflammation [8].

Systematic research on acute experimental cord injuries dates back as early as 1914 when Allen [9] studied the impact of dropping metallic weights on the spinal cord in dogs at specified time intervals [7, 10]. After these first crude experiments, research focused on the understanding of the pathophysiology and possible treatments of ASCI.

Pathophysiology

Nowadays, it is accepted that acute spinal cord injury involves a two-step process of primary and secondary injury [7, 8, 11, 12]. According to this concept, the primary injury involves trauma to the cord itself, or to the surrounding vertebral column [11]. This

initial impact might be due to fracture dislocation, burst fractures, missile injuries, or acutely ruptured discs followed by a persisting contusion and compression of the area [7]. Distractional forces associated with flexion, extension, or rotation can also result in stretching and shearing of the neural elements themselves, or of the spinal cord vasculature [11]. It must be kept in mind that the extent of the secondary injury also depends on the location of the primary injury. The larger spinal canals of the cervical region might provide a buffer for any applied mechanical stress, while the narrower canals of the thoracic spine lack this protection. Therefore, injuries in the thoracic region tend to be more severe, leading more often to complete spinal cord injuries than cervical or lumbar spine injuries [7, 11].

Secondary injury develops during the first few hours to days after the primary insult. Systemic effects include hypotension and reduced cardiac output [13]. The mechanisms of the local effects of secondary injury involve an entire cascade of biochemical and cellular processes leading to cellular damage or even cell death [7, 13]. Figure 21 shows the many mechanisms involved in secondary injury. Those mechanisms include vascular changes such as ischemia, impaired autoregulation, neurogenic shock, hemorrhage, microcirculatory derangements, vasospasm, and thrombosis; ionic derangements such as increased intracellular calcium, increased extracellular potassium, and increased sodium permeability; neurotransmitter accumulation, including serotonin or catecholamines, and extracellular glutamate (the latter responsible for causing excitotoxic cell injury); arachidonic acid release and free radical production, eicosanoid production, and lipid peroxidation; endogenous opioids; edema; inflammation; loss of

adenosine triphosphate-dependent cellular processes; and programmed cell death (also known as apoptosis) [7, 8, 11, 13].

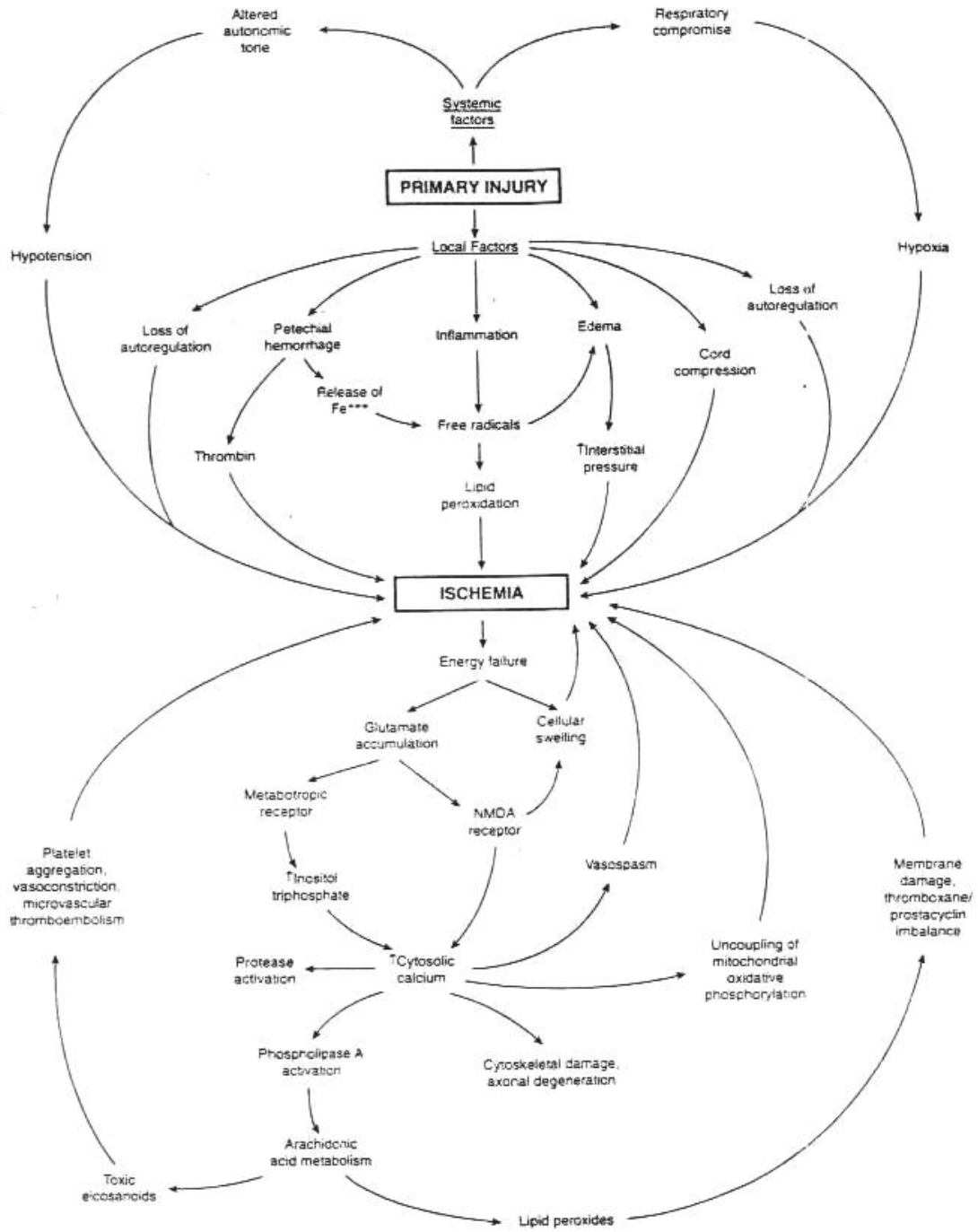


Figure 2-1. Mechanisms in the pathogenesis of ASCI [Amar, A.P. and M.L. Levy, *Pathogenesis and pharmacological strategies for mitigating secondary damage in acute spinal cord injury.* Neurosurgery, 1999. **44**(5): p. 1027-39; discussion 1039-40]

Immediately after the primary injury, petechial hemorrhages in the spinal cord develop (due to ruptures of postcapillary venules or arterioles caused by the mechanical distress itself) [10, 11, 13]. In addition to bleeding, edema, release of vasoactive substances, and thrombosis (caused by platelet aggregation) lead to a dramatic reduction in spinal cord blood flow [7, 8, 10, 11, 13-15]. The loss of autoregulation after ASCI further decreases the spinal cord blood flow, and worsens the ischemia. If left untreated, this ischemia worsens over the first few hours [7, 11, 13].

Ischemia initiates secondary endogenous excitatory amino acid (EAA) neurotransmitter-dependent mechanisms [7, 11, 13]. Depletion of the adenosine-5'-triphosphate (ATP) supply leads to a malfunctioning of the sodium-potassium ATPase, which is responsible for cellular homeostasis [11, 16]. This process is followed by an uncontrolled efflux of potassium; and influx of sodium, chloride and calcium. The large calcium influx activates several calcium-dependent processes (such as activation of phospholipase A2, mobilization of fatty acids, synthesis of toxic eicosanoids, generation of free radicals, further decrease of energy reserves, axonal degeneration, and activation of lytic enzymes) [11, 14, 17, 18].

Another factor that plays a major role is the formation of free radicals such as lipid peroxides (a by-product of the cyclooxygenase pathway), which is a result of the phospholipase A2 activation [7, 11, 16]. The formation of lipid peroxides spreads over the entire cell membrane, destroying the structural and functional integrity of the cell membrane, and finally causing cell death.

A form of programmed cell death (known as apoptosis) can also occur [7]. In a rat experiment Springer et al. [19] showed that the upstream and downstream components of

the caspase-3 pathway are activated after traumatic spinal cord injury. Caspase-3 belongs to the cysteine proteases, that regulate the execution of apoptotic cell death in mammals [19]. These findings were confirmed in the human spinal cord by Emery et al. [20].

Treatment

As pharmacological treatment of the primary injury is not possible, treatment mainly involves stabilization of the acutely injured patient (especially his cardiopulmonary status). To prevent systemic hypotension and hypoperfusion, which may result in neurogenic shock, treatment focuses on fluid administration and vasopressor support [12].

Pharmacological treatment of the secondary injury has been subject to intensive research, and many of the biochemical and cellular processes have been targeted [8, 12, 16, 21]. Our focus is the role of corticosteroids in the treatment of acute spinal cord injury.

Over 50 years ago, glucocorticoids were introduced as drugs for the treatment of inflammatory diseases [22]. Studies in the 1960s showed a beneficial effect in treating certain neurological disorders, including brain tumors [23]. Therefore, the rationale for using glucocorticoids in the treatment of acute spinal cord injuries, to reduce spinal cord edema, was based on their ability to reduce peritumoral cerebral edema [12, 23]. Even though the results showed only moderate positive effects, corticosteroids have rapidly received special attention in treating patients with acute spinal cord injury [23, 24]. Over the following years glucocorticoids were extensively investigated in pre-clinical and clinical studies to elucidate their beneficial effects [12, 16, 23, 25, 26]. Results of animal experiments proposed numerous effects. In addition to edema prevention, other positive effects were suggested, such as enhanced spinal cord blood flow, stabilization of

membrane structures, alteration of electrolyte concentrations in the injured tissue, facilitation of neuronal excitability and impulse generation, protection from free radicals and inhibition of the inflammatory response [6, 11, 16, 27-29]. Among all corticosteroids, methylprednisolone has become the steroid of choice and has been studied extensively in clinical trials [12]. Braughler and Bracken found that methylprednisolone in comparison with other steroids seems to possess better antioxidant characteristics, passes cell membranes faster, and inhibits the neutropenic response to activated complement components more effectively [12, 25, 30].

Because of the many positive outcomes in pre-clinical studies, methylprednisolone was studied in clinical trials. In 1979, the National Acute Spinal Cord Injury Study (NASCIS 1) was the first of three large clinical trials evaluating the efficacy of methylprednisolone in human SCI [6, 12]. In this study a methylprednisolone IV bolus dose of 100 mg followed by 100 mg daily for the next 10 days was compared to a high dose of 1000 mg IV bolus followed by 1000 mg daily for the next 10 days. Three hundred thirty acutely injured patients were enrolled in this study and the neurological recovery was assessed 6 weeks and 6 months after injury. The study was conducted without placebo control because the investigators believed that methylprednisolone shows efficacy, and withholding therapy would have been unethical [6, 12]. The outcome of this study was rather disappointing, because it did not provide any evidence for the efficacy of methylprednisolone. Patients treated with the high dose of 1000 mg daily had nearly identical neurological recovery of motor and sensory function at the 6 weeks and 6 months check-up [6, 25]. Furthermore, the high-dose regimen was associated with an increased risk of side effects, such as wound infection, pulmonary emboli, or even death

[23, 25]. However, data from animal studies conducted after conclusion of NASCIS 1 suggested that the administered dose of methylprednisolone was too low to induce an inhibition of lipid peroxidation. They also showed that the start of treatment plays a crucial role, and treatment needs to be initiated as early as possible after the injury [12, 23, 25, 31]. With those results in mind, a multicenter, randomized, double-blind, placebo-controlled study (NASCIS 2) was initiated in 1985 to evaluate the efficacy of methylprednisolone at a much higher dose (30 mg/kg IV bolus followed by 5.4 mg/kg/h infusion for 23 h), and the efficacy of the opioid receptor antagonist naloxone (5.4 mg/kg IV bolus followed by 4 mg/kg/h infusion for 23 h) [6]. Results of this study were reported in 1990. Analysis of all the data did not reveal a difference between the treatment groups [23]. However, a *post hoc* statistical analysis showed that administering methylprednisolone within 8 h after injury significantly improved neurological recovery 6 months and 1 year after the injury, when compared with methylprednisolone administered after 8 h, naloxone, and placebo [6, 12, 23].

Results of the third study (NASCIS 3) were reported in 1997. This double-blind, randomized, nonplacebo controlled study determined the effect of the duration of methylprednisolone therapy. The first goal was to determine whether methylprednisolone given over 48 h (30 mg/kg IV bolus followed by 5.4 mg/kg/h for 48 h) significantly improves neurological recovery when compared to 24 h (30 mg/kg IV bolus followed by 5.4 mg/kg/h for 24 h). The second objective was to evaluate the efficacy of tirilazad mesylate, a 21-aminosteroid, in the treatment of ASCI [11, 12]. The study found that if treatment is initiated within 3 h after injury, extending the methylprednisolone infusion beyond 24 h did not enhance recovery, whereas if treatment is started between 3 and 8 h

methylprednisolone infusion should be extended to 48 h [11, 12]. Infusion of the non-glucocorticoid tirilazad mesylate for 48 h showed similar effect as the 24 h infusion of methylprednisolone, but it was inferior to the 48 h regimen [11, 12]. The outcome of this study was used to support the use of high-dose methylprednisolone in spinal cord injury as a standard of care.

Despite the implication of positive results of these trials, there is still a controversy about the efficacy of methylprednisolone in the treatment of acute spinal cord injury and its ability to improve neurological outcome [8, 12, 32-37]. Several flaws in the study design, the reporting, and data analysis have been reported [8, 11, 12, 32, 33]. The drawback of NASCIS 1 was that the study was approved and conducted without a control group. Without a placebo group, it could not be determined if methylprednisolone has a beneficial or harmful effect on recovery from SCI. There were also no clear inclusion criteria (such as a minimum motor impairment). Therefore, patients with no or only minimal motor deficits were included as well [6].

The NASCIS 2 study did not specify these inclusion criteria either. When the entire population of NASCIS 2 was analyzed, a clinical difference between the two treatment groups could not be shown [23]. Only *post hoc* analyses could detect a small increase in neurological recovery in a subgroup of patients that had received methylprednisolone within 8 h of injury [38]. Furthermore, the number of patients included in the subgroup from which a positive effect of methylprednisolone was detected constituted only a small percentage of the total number of patients. Medical and surgical protocols differed among the different testing sites [11]. One of the biggest failures was that no outcomes of neurological recovery were measured. Therefore, it is difficult to determine if the changes

in motor scores are related to clinical improvements of the patients [6, 11, 12, 32]. Even more confusing was the report of change in motor scores for patients with incomplete SCI in the placebo group. Patients receiving placebo more than 8 h after the injury showed significantly better neurological recovery than patients in the group receiving placebo within 8 h. It was also surprising that the neurological outcome of patients treated with placebo more than 8 h after injury was almost identical to that of patients treated with methylprednisolone within the 8 h time window [6, 33].

Another point of criticism was that the baseline groups were not comparable, and proper statistical analysis not possible [32]. Statistical procedures were subject to criticism as well. They included no report of raw data, problematic statistical comparisons, simplification of subgroup analysis, *post hoc* analysis of only subgroups of the entire study population, and interpretive errors [6, 32, 33].

Similar criticism arose for the NASCIS 2 and the NASCIS 3 study. A positive effect of methylprednisolone could again only be shown in a *post hoc* analysis of a subgroup of patients, where infusion was extended to 48 h after start of treatment (between 3 and 8 h after injury). As patients within this group had a higher incidence of severe pneumonia, sepsis, and death from respiratory complications, clinical significance of improvements in neurological recovery are questionable [6, 8, 33, 38].

Although corticosteroids, especially methylprednisolone, have been used to treat acute spinal cord injury for a long time, evidence for their beneficial effects is limited. Further studies are required to determine the efficacy. Until then methylprednisolone should be used with care as a treatment option, but not a standard of care [38].

Nonlinear Pharmacokinetics of Prednisolone

Prednisolone (a potent synthetic corticosteroid that became available for clinical use in 1955) is widely used in the management of a variety of diseases including congenital adrenal hyperplasia; severe asthma; certain hematological disorder; and rheumatic, gastrointestinal, and malignant diseases [39, 40]. Despite its advantages, prednisolone therapy has been also associated with a pronounced side effect profile [41, 42]. Therefore, a better understanding of the pharmacokinetics of prednisolone might be helpful in optimizing drug therapy. Since then, the pharmacokinetics of prednisolone have been subject to considerable research. However, the results of different groups are conflicting [40, 43-45]. A number of studies found a dose-dependency of the pharmacokinetic parameters, while others were unable to confirm them [39, 46-48].

In 1977, Pickup et al. [39] studied the elimination of prednisolone after intravenous administration to volunteers and arthritic patients. Their study was based on variable elimination half-lives reported by several groups [39]. In general, with increasing dose, the half-lives were found to be longer and the volume of distribution and clearance were larger. They proposed that the dose-dependency is due to saturable protein binding [39]. The nature of the interaction between prednisolone and serum proteins has been intensively studied [44, 46, 47, 49]. It could be shown that prednisolone binds to two plasma proteins, albumin and the α_1-glycoprotein transcortin (corticosteroid binding globulin, CBG) [39, 40, 42, 46, 47, 49]. Binding to albumin is characterized by low affinity and high capacity; whereas transcortin binding is believed to be of high affinity, but low capacity [39, 42, 47, 49]. Because of the low capacity, transcortin is easily saturated at higher concentrations. Therefore, the fraction bound of prednisolone decreases with increasing concentration. This will result in an alteration in the

pharmacokinetics of prednisolone, as the unbound plasma concentration is considered to be the active drug [42]. The increase of the free fraction at higher concentrations is also reflected in a greater clearance and larger volume of distribution, as determined by Pickup et al. [39].

Rose et al. [46] could confirm these findings. It could be shown that the systemic clearance and volume of distribution varied in a dose-dependent manner, when evaluated as a function of total drug concentrations. The protein binding was concentration-dependent also, and was characterized by binding to albumin and transcortin. The parameter of the free prednisolone in plasma followed linear pharmacokinetics. The evaluation of the pharmacokinetics and pharmacodynamics in man by Wald et al. [44] provided similar results. They determined the kinetics and dynamics of prednisolone after IV bolus administration of two different doses, and found increases in clearance and volume of distribution, based on total plasma concentrations. The values of protein binding were consistent with those found by Rose et al. [44, 46]. But unlike the findings of Rose and collegues, Wald et al. found the pharmacokinetics of the free drug to be slightly nonlinear, as indicated by a decrease in clearance and volume of distribution values. Wald et al. proposed that this nonlinearity is due to time-dependent changes, as shown in earlier studies by Meffin et al. [50] and English et al. [51]. In 1992, Barth et al. [45] also found a diurnal variation in the pharmacokinetic parameter of prednisolone. In all three studies, the concentrations in plasma were higher after administration in the morning. The diurnal variation may be a result of the circadian rhythm of endogenous cortisol. The higher plasma concentration levels of cortisol in the

morning could cause an inhibition of the prednisolone clearance and protein binding, increasing its concentration [50].

Despite that, there are studies that could not find any dose- or time-dependence of prednisolone pharmacokinetics [48, 52]. In two studies, conducted by Tanner et al. [52] and Al-Habet et al. [48], prednisolone pharmacokinetics was determined after IV bolus or oral administration of different doses. Both groups found no change in the terminal half-life of prednisolone over the dose range studied. However, Tanner et al. [52] reported an increase in the volume of distribution and clearance, which could not be accounted for by changes in protein binding. Since there was no alteration in plasma protein binding, they suggest that the dose-related increase might be due to an increase in tissue binding or saturation of glucocorticoid receptors.
Al-Habet et al. [48] on the other hand, did not find a dose-related increase in volume of distribution or clearance. Their data also showed proportionality between dose and the area under the curve (AUC). Therefore, they could not confirm any nonlinear pharmacokinetic behavior of prednisolone.

Another factor contributing to the nonlinear pharmacokinetics based on saturable protein binding is competition for the transcortin binding sites between endogenous cortisol and prednisolone [41, 43, 53, 54]. Such competition may alter the pharmacokinetics of these compounds by changes in the free fraction in plasma [43]. Rocci et al. [43] could show that prednisolone binding to transcortin is saturable, and that there is a substantial displacement of prednisolone from transcortin binding sites in the presence of cortisol, resulting in a larger fraction unbound. Therefore, the degree of prednisolone bound to transcortin depends on the concentration of endogenous cortisol.

The situation gets even more complex when the suppression of endogenous cortisol by prednisolone is taken into account. Simulations by Rohatagi et al. [54] take all these considerations into account, offering a good estimation of free prednisolone concentrations. The competition between those two compounds might be of clinical importance because free, unbound prednisolone concentrations will be systematically underestimated if the influence of endogenous cortisol is ignored. From the above, it can be concluded that the pharmacokinetics of prednisolone are very complex. Therefore, it is difficult to optimize dosing regimens, and make accurate predictions of the expected effect-time relationship.

Corticosteroids in the Treatment of Asthma and Allergic Rhinitis

Bronchial asthma and allergic rhinitis are among the most common chronic diseases of modern society. Despite recent advances in drug therapy, the incidence is still increasing. Asthma is a chronic inflammatory disorder of the airways that involves many cells and cellular elements. The chronic inflammation causes an associated increase in airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing (particularly at night or in the early morning). These episodes are usually associated with widespread but variable airflow obstruction, which is often reversible, either spontaneously or with treatment [55].

To an increasing degree, asthma is a major cause of illness, hospitalization, loss of productivity, and death. The main goals of asthma therapy are to prevent chronic symptoms, to maintain nearly normal pulmonary function, to maintain normal activity levels, and to prevent recurrent asthma exacerbations and minimize the need for hospitalizations [56].

Allergic rhinitis is caused by an inflammation within the nose in response to allergy triggers, such as pets and pollens. It leads to the typical symptoms of nasal congestion, sneezing, runny and itchy nose, and itchy, watery eyes [57]. There are two different forms of allergic rhinitis: seasonal and perennial allergic rhinitis. Seasonal allergic rhinitis is triggered mainly by natural pollen exposure, while perennial allergic rhinitis may be caused by various environmental allergens [58]. Suffering from allergic rhinitis results in a loss of productivity, and also results in a generally impaired health-related quality of life.

Over the years, drug therapy of asthma and allergic rhinitis has been significantly improved, mainly by the introduction of new corticosteroids with better pharmacokinetic properties (and therefore, improved therapeutic ratios).

Inhaled and intranasal corticosteroids are the drugs of choice in the therapy of asthma and allergic rhinitis. However, there has recently been an increased awareness of their ability to produce systemic adverse effects. The availability of more potent corticosteroids and new delivery systems has focused attention on these safety issues [59]. It is the goal of all inhaled and intranasal corticosteroids to (1) produce long-lasting therapeutic effects at the site of action, (2) minimize oral availability, and (3) minimize systemic side effects by rapid clearance of absorbed drug. Currently, 6 inhaled corticosteroids are available for the treatment of asthma and/or allergic rhinitis, and others are in development. The 6 available are triamcinolone acetonide[1], flunisolide, beclomethasone dipropionate, budesonide, fluticasone propionate, and

1 B=beclomethasone, BDP=beclomethasone dipropionate, BMP=17-beclomethasone monopropionate, BUD=budesonide, CIC=ciclesonide-prodrug, DES-CIC=des-ciclesonide, active metabolite of CIC, FLU=flunisolide, FP=fluticasone propionate, MF=mometasone fuorate, TA=triamcinolone acetonide, LE=loteprednol etabonate

mometasone fuorate. Ciclesonide and loteprednol etabonate are still in development for inhaled and intranasal use, respectively. We review their pharmacokinetic and pharmacodynamic properties, and consider the necessary properties of an ideal topical corticosteroid for use in the treatment of asthma and allergic rhinitis.

Pharmacodynamic Properties

Receptor binding

The pharmacodynamic properties of corticosteroids can be described by the binding of the drug to its receptor because the pharmacological effect of corticosteroids is mediated through the glucocorticoid receptor. Due to the ubiquitous nature of the glucocorticoid receptor, corticosteroids act on a wide variety of cell types. This accounts for their systemic effects, in addition to their local therapeutic effects. The glucocorticoid receptor is located in the cytoplasm. Although there are two different types of glucocorticoid receptors, currently available glucocorticoids only bind to the type II receptor (the type I is also known as the mineralocorticoid receptor). The type II receptor is expressed in almost all tissues and cells and the pharmacological, both beneficial and unwanted, effects of inhaled and intranasal corticosteroids are mediated through reversible binding to this receptor. After binding to the receptor, the drug-receptor-complex translocates into the nucleus, binds to DNA and hence, activates or represses gene transcription through different mechanisms. Recent research focuses on finding new glucocorticoids, that will separate these transactivation and transrepression processes [1, 22].

Transactivation, stimulation of gene transcription, was found to be correlated with several negative side effects of corticosteroids, while transrepression, repression of transcription factors such as NF-κb and AP1, seems to be responsible for the

anti-inflammatory effect [60]. Important for the stimulation of transcription is dimerization of the ligand-bound receptor. Dimerization is both necessary for high affinity binding of the receptor to the glucocorticoid response element (GRE) and glucocorticoid-dependent induction of gene transcription [22, 60, 61]. Negative regulation by glucocorticoids can be achieved by either a direct interaction of the glucocorticoid receptor with a site on the DNA called negative glucocorticoid response element (nGRE) or via protein-protein interactions such as AP1 and NF-κb [1, 60, 61]. This new approach could be very promising in increasing the therapeutic index but further research needs to be done to prove this concept.

Depending on their receptor binding affinity, different drugs have different potencies. Potency is an important measure of pharmacological action as higher receptor affinity is associated with an increased pharmacological response [62]. However, increasing the potency of a glucocorticoid will not necessarily increase the therapeutic ratio (topical: systemic activity) as increasing the potency will lead to higher topical efficacy but at the same time also more systemic activity and a higher incidence of systemic side effects.

Table 2-1 and Figure 2-2 show relative receptor binding affinities for different corticosteroids used for inhalation and intranasal use [63, 64]. The receptor binding affinities are usually given in comparison with an affinity of 100 for the standard dexamethasone. MF is reported to have the highest receptor binding affinity (2300), followed by FP (1800) and 17-BMP (1345) [64, 65]. The active metabolite of the new corticosteroid ciclesonide, des-ciclesonide, shows also a high receptor binding affinity (1200) while LE is reported to have a receptor binding affinity of 430 [66, 67]. As

receptor binding affinity can be compensated by administering dose equivalents, the pharmacokinetic properties of the corticosteroids are the more important factors to evaluate their safety and efficacy [68].

Table 2-1. Pharmacokinetic and pharmacodynamic parameters of inhaled and intranasal corticosteroids

Corticosteroid	RRA	F_{oral} %	fu %	CL L/h	Vd_{ss} L	$t_{1/2}$ elim. h	Ref.
MF	2300	< 1	1-2	54	—	5.8	[63, 65, 69, 70]
FP	1800	< 1	10	66-90	318-859	7-8	[64, 71-75]
BDP	53	15-20	13	150	20	0.5	[64, 76-78]
17-BMP	1345	26	—	120	424	2.7	[64, 76]
B	76	—	—	—	—	—	[64]
CIC	12	< 1	< 1	152	207	0.36	[66, 79, 80]
Des-CIC	1200	< 1	< 1	228	897	3.4	[66, 79, 80]
BUD	935	11	12	84	183-301	2.8	[64, 81, 82]
LE	430	—	10*	63*	37*	2.8*	[67, 83]
TA	233	23	29	37	103	2.0	[64, 84, 85]
FLU	180	20	20	57	96	1.3	[64, 86-88]

RRA = relative receptor affinity, F_{oral} = oral bioavailability, fu = fraction unbound, CL = clearance, Vd_{ss} = volume of distribution at steady-state, $t_{1/2}$ = half-life, Ref. = reference
* in dogs

Pharmacokinetic Properties

Prodrug

A prodrug is a pharmacologically inactive compound that is activated in the body after its administration. To exert a local effect, a prodrug needs to be activated in the target tissue (e.g. lung or nose). For corticosteroids used in inhalation and intranasal therapy, activation is usually initiated by esterases in the lung/nose. This tissue selectivity also reduces the risk of local and systemic side effects if the esterases necessary for the activation are mainly found in the lung tissue.

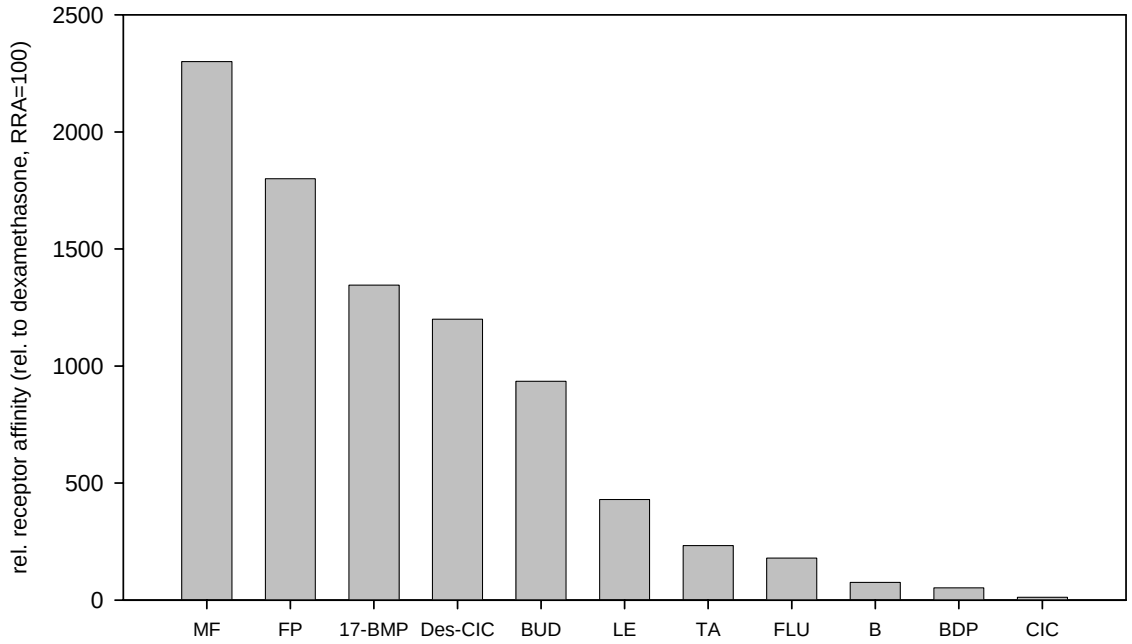


Figure 2-2. Relative receptor binding affinities of inhaled and intranasal corticosteroids

After inhalation of an active corticosteroid, a part of the drug is deposited in the mouth and oropharynx leading to side effects such as oral candidiasis. Administration of an inactive prodrug may reduce the incidence of local side effects in the mouth and oropharynx region, since the inactive drug deposited in that region will be swallowed before activation can occur. This concept has been proven in studies on ciclesonide. Administration of the prodrug ciclesonide resulted in significantly less oral deposition of ciclesonide and its active metabolite des-ciclesonide than fluticasone propionate [Richter, K., Nielsen-Gode, D., Biberger, C., Nave, R., and Magnussen, H., *Oropharyngeal deposition of inhaled ciclesonide and fluticasone propionate in asthmatics*. Poster, 2002, ERS meeting in Stockholm, Sweden, September].

Desired characteristics of a prodrug are essentially no receptor binding, rapid hydrolysis to the active compound in the lung or nose, high receptor binding affinity of the active compound and no pharmacological effect of its secondary metabolites [68]. Besides all the advantages of using inactive prodrugs, it has to be ensured that the prodrug actually is converted to the active drug after administration to avoid failure of therapy. Beclomethasone dipropionate is, so far, the only corticosteroid on the market used in the treatment of asthma and allergic rhinitis that has a prodrug structure. BDP has a low receptor binding affinity (RRA=53) whereas its active form, 17-beclomethasone monopropionate, binds to the glucocorticoid receptor with a high affinity (RRA=1345). 17-BMP is further metabolized to beclomethasone that has a receptor binding affinity lower than dexamethasone (RRA=76) (Table 2-1). A second drug with a prodrug structure, which is still in development for the treatment of asthma, is ciclesonide. It is administered as the prodrug and activated by esterases in the airways [Hochhaus, G., Talton, J., and Stoeck, M., *Pulmonary targeting of ciclesonide and its active metabolite as determined in an ex-vivo rat receptor-binding assay*. Poster, 2002. ERS meeting, Stockholm Sweden, September]. The parent compound is 100-fold less potent in binding to the glucocorticoid receptor than the active principle des-ciclesonide.

Soft-Drug

Another way to target a drug is the softdrug concept. A softdrug is essentially the opposite of a prodrug. It is defined as a biologically active chemical compound characterized by a predictable and controllable *in vivo* metabolism to nontoxic moieties, after they achieve their therapeutic role [4]. In short, the softdrug is active *per se* while the prodrug is inactive *per se*. The inactivation occurs ideally in a single step, although further metabolism of the inactive metabolite is possible [89]. The metabolic inactivation

occurs preferably through simple steps (e.g. hydrolysis), without the use of enzymes that could be saturated. There are several approaches to design soft drugs. One example of the inactive metabolite approach is loteprednol etabonate. It was designed by using the known inactive metabolite cortienic acid of hydrocortisone as a lead compound. Appropriate structural changes led to the active compound loteprednol etabonate that is again inactivated in a single metabolic step.

Bioavailability

The pharmacokinetic properties of the corticosteroid as well as the application device and technique determine how much steroid will reach the sites of desired and undesired activity and how long it will stay there. Important aspects are bioavailability and drug formulation.

The bioavailability of an inhaled/intranasal corticosteroid is the rate and extent at which the drug reaches its site of action (pulmonary/nasal bioavailability) and the blood (systemic bioavailability).

After inhalation, a large part (approximately 40-90%) of the dose is swallowed and then available for systemic absorption. This bioavailability of the orally delivered part is dependent on absorption characteristics of the drug from the gastro-intestinal tract and the extent of intestinal and hepatic first-pass metabolism. Since the orally absorbed fraction of the drug does not contribute to the beneficial effects but can induce systemic side effects, it is desirable for the oral bioavailability of inhaled corticosteroids to be very low.

The oral bioavailabilities of currently used corticosteroids range from less than 1% for fluticasone propionate to 26% for 17-beclomethasone monopropionate [70, 71, 76, 77, 81, 84, 86], Nave, R., Bethke, T., van Marle, S.P., and Zech, K., *Pharmacokinetics of 14C-ciclesonide after oral and intravenous administration in healthy subjects*. Poster,

2002. ERS Meeting in Stockholm, Sweden, September]. However, the main determinant of systemic bioavailability after inhalation is direct absorption from the lung, where for the currently available inhaled corticosteroids there is no first-pass effect. All of the drug that is deposited in the lung will be absorbed systemically [59]. The percentage of the dose that is deposited in the lung is greatly influenced by the efficiency of the delivering device. The pulmonary bioavailability is rather a function of the delivery device used for inhalation than a property of the drug itself. The pulmonary bioavailability will depend on the amount deposited in the lungs and will differ with the delivery device used [59, 90]. Fluticasone propionate, for example, has an oral bioavailability of <1% due to a high first-pass metabolism. When administered to the lungs using a dry powder inhaler (DPI), the absolute bioavailability (systemic + pulmonary) is reported to be approximately 17%, compared to 26% to 29% when using a metered dose inhaler (MDI) [91-93]. After mometasone fuorate administration via a dry powder inhaler the absolute bioavailability was reported to be 11% [91]. Table 2-2 summarizes the device-dependent parameters of corticosteroids such as the pulmonary and nasal bioavailabilities after inhalation and intranasal administration, respectively. Table 2-1 shows the systemic bioavailability after oral administration [94].

Most of what was said for inhaled administration is also true for intranasal use. A major advantage of intranasal delivery is, that the efficiency of deposition is usually high (about 80%) [63]. However, following intranasal delivery there is a rapid mucociliary clearance from the nose into the throat and consequently a large proportion of the dose (~70%) is swallowed, which in turn can be responsible for systemic side effects after absorption from the gastro-intestinal tract [59].

There are several factors influencing the degree of systemic bioavailability such as droplet size of a liquid formulation, the particle size of a suspension, the type of formulation (solution/suspension), the delivery device and the physico-chemical properties of the drug itself [63].

Table 2-2. Device-dependent bioavailability after inhaled and intranasal administration of corticosteroids

Corticosteroid	$F_{inhaled}$ %	Device	F_{nasal} %	Device	Ref.
MF	11	DPI-MDI	—	—	[91]
FP	17	DPI	—	—	[91-93]
	26	CFC-MDI			
	29	HFA-MDI			
17-BMP	36	CFC-MDI	44	aq.nasal spray	[76]
Des-CIC	52	HFA-MDI	—	—	[95]
BUD	18	CFC-MDI	~66	powder inhaler	[73, 82, 96]
TA	22	CFC-MDI	—	—	[84]
FLU	20	CFC-MDI	—	—	[97]
	68	HFA-MDI			
BDP	55-60	HFA-MDI	~0	aq.nasal spray	[76]

$F_{inhaled}$ = bioavailability after inhaler, Device = inhaler device, F_{nasal} = bioavailability after intranasal administration, Device: intranasal device, Ref. = Reference

For example, it could be shown that the bioavailability of fluticasone propionate is increased eightfold if an aqueous nasal spray is used compared to nasal drops. However, the bioavailabilities for both formulations were low with 0.51% for the spray and 0.06% for the drops [98]. The percentage of the dose that is swallowed is dependent on the lipophilicity of the drug. A high degree of lipophilicity diminishes water solubility and therefore increases the amount of drug swept away by nasociliary clearance before it can get access to the receptor sites [59]. Therefore, a high degree of lipophilicity might not be favorable for a drug used for intranasal application because sufficient drug needs to be dissolved and absorbed into the target cells in the nasal mucosa to be effective. Estimated

absolute bioavailabilities for corticosteroids after intranasal administration vary from 49% for flunisolide to 44% for beclomethasone dipropionate to 34% for budesonide to less than 1% for fluticasone propionate and mometasone fuorate [76, 99-101]. However, differences in systemic bioavailability may also arise from different delivery devices. In general, aqueous solutions seem to have higher intranasal bioavailabilities than dry powders or pressurized aerosols [102].

Drug formulation

Another important factor in assessing the efficacy and safety of an inhaled/intranasal corticosteroid is the delivery device. Inhaled corticosteroids are administered either via a metered-dose inhaler (MDI) or via a breath-activated dry powder inhaler (DPI). The MDI contains the drug either as a suspension in a carrier liquid or a solution delivered through a chlorofluorocarbon (CFC) or hydrofluoroalkane (HFA) propellant, respectively, although CFC-MDIs are gradually phased out because of their ozone-depleting potential [92]. Additional to their environmentally friendly property, HFA solutions also seem to have the advantage of delivering a much greater mass of fine particles. Fine particles, with a diameter of less than 5 μm, are more likely to be deposited in the tracheo-bronchial and pulmonary regions in the lung. Larger particles on the other side, are deposited mostly in the oropharynx where they are swallowed and increase the risk of systemic absorption [103]. The average particle diameter delivered by a CFC-MDI is 3.5-4.0 μm whereas the average particle diameter delivered by a HFA propellant is around 1.1 μm. This difference in particle diameter might have a clinical significance as the average diameter of small airways is around 2 μm, resulting in a greater lung deposition [104]. This increased proportion of fine particles with the HFA-MDI results in an improved lung deposition. In a study using inhaled

beclomethasone dipropionate, the lung deposition increased from 4-7% with CFC-BDP to 55-60% for a newly developed HFA-MDI formulation [105]. A high lung deposition was also found for ciclesonide. With the CFC-free solution MDI, a mean lung deposition of 52% could be obtained [Bethke, T.D., Boudreau, R.J., Hasselquist, B.E., Davidson, P., Leach, C.L., Drollmann, A., Hauns, B., and Wurst, W., *High lung deposition of ciclesonide in 2D and 3D imaging*. Poster, 2002. ERS meeting, Stockholm, Sweden, September]. In a single-dose study comparing HFA flunisolide and CFC flunisolide, the drug deposition in the lung could even be increased to 68% (HFA) compared to 19.7% (CFC) [95].

Lung deposition can also be increased by use of spacer devices, which can alter the amount of fine particles and therefore, increase the respirable fraction and decrease the amount of drug deposited in the oropharynx [97]. However, it also needs to be kept in mind that a greater lung deposition might result in a greater possibility of systemic adverse effects because of the lack of first-pass metabolism after direct absorption from the lung. The other inhaler type used for inhalation of corticosteroids is the dry powder inhaler. The DPI offers an easier delivering technique, which requires less coordination than the MDI. However, it requires a forceful deep inhalation to trigger the inhalation device to help break up the aggregates of the micronized powders into respirable particles in the oropharynx and larger airways. Thus, lung deposition is flow-dependent and the higher the inhalation flow, the smaller the particles will be [106]. An inspiratory flow of 60 L/min is considered to be optimal [97]. Therefore, the flow characteristic should be determined and it should be ensured that asthmatic patients in all asthma stages are able to achieve an inhalation flow that is enough to achieve the required effect [106]. In a lung

deposition study with budesonide it could be shown that reducing the inhalation flow from 58 L/min to 36 L/min reduces the lung deposition from around 28% to around 15% [107].

There have been also new developments in the field of nebulizers and liquid formulations. Among those are the inhalation device Mystic™ from Batelle, which is based on electro-hydrodynamic principles, using electrostatic energy to create fine aerosols from formulated drug solutions or suspensions thereby increasing the pulmonary tract deposition to about 80% [108] and the RESPIMAT device from Boehringer Ingelheim, which uses a high-pressure micro-spray system of nozzles to release a metered dose to the patient. This system generates a slow release of the drug with a high concentration of respirable particles [109].

There are also differences among the delivery devices for nasal administration. Currently there are three different devices on the market, pressurized metered dose inhaler (pMDI), aqueous pump spray and powder. The aqueous pump spray and the powder formulations are preferred, because they offer a better intranasal distribution than the pressurized aerosols [110]. Compared to inhaled corticosteroids, the efficiency of intranasal drug deposition is not one of the main concerns with the devices currently in use because it is in general very high (around 80%) [63]. For budesonide, for example, the intranasal bioavailability is reported to be around 66% using a dry powder inhaler [96]. However, after administration, a large portion of the dose is transported into the gastrointestinal tract by nasal mucociliary clearance [111]. To avoid systemic side effects from the swallowed part, a low oral bioavailability and a high clearance are desirable characteristics of the drug (see bioavailability). As with inhaled corticosteroids the

absolute bioavailability of an intranasally administered drug is the sum of the orally absorbed portion and the portion that is absorbed directly from the nose into the systemic circulation. Absorption across the nasal mucosa varies significantly and increases with increasing water solubility of the drug [111]. Therefore, highly lipophilic drugs, such as fluticasone propionate, have diminished water solubility in the nasal mucosa and increase the amount of drug swept away by mucociliary clearance before it can reach the receptor sites [59]. The physico-chemical state of the formulation is another important factor influencing the local and systemic concentration after nasal application [63]. It could be shown that after administration of a solution-based triamcinolone acetonide product absorption is faster than after an aqueous suspension of the same drug. This might increase the local and the systemic concentrations after intranasal application [112].

Finally, delivery of the drug to the lung or nose does not only depend on the device itself but also on the patient because every inhaler or nasal spray require a certain technique for optimal drug delivery [97].

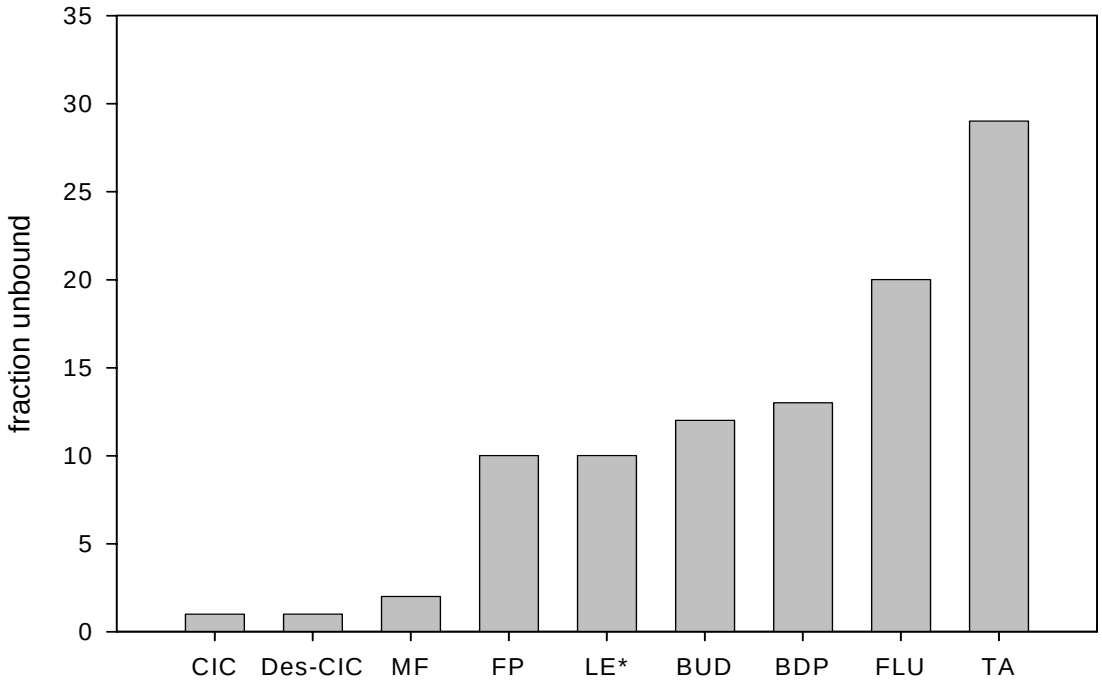
Protein binding

Many drugs are bound to plasma proteins once they reach the systemic circulation. Binding to plasma proteins, such as albumin and transcortin, keeps the drug in the blood stream and prevents its diffusion into the tissue. Most of the synthetic corticosteroids are moderately to highly protein bound (≥70%). Since it is understood that only the free, unbound drug is pharmacologically active, knowledge about the protein binding might be important in assessing the pharmacokinetics and pharmacodynamics of a drug. A high plasma protein binding will consequently lead to a low fraction unbound and suppression of endogenous cortisol, an important measure of systemic side effects, might become

insignificant. In case of linear protein binding the plasma concentration of the free drug is a constant fraction of the total drug. However, if nonlinear protein binding occurs this issue becomes more difficult because the fraction of the unbound drug is not constant [53]. Corticosteroids used for inhalation or intranasal use show linear protein binding to albumin. However, the extent of binding differs, with ciclesonide showing the highest degree of binding (99%) [79], followed by mometasone fuorate (98-99%) [69], 17-beclomethasone monopropionate (98.4% in rat plasma) [113] and fluticasone propionate (90%) [72], budesonide (88%) [81] and beclomethasone dipropionate (87%) [78]. Flunisolide and triamcinolone acetonide show a lower protein binding with fractions bound of 80% [87] and 71% [85], respectively (Figure 2-3, Table 2-1). For the new corticosteroid loteprednol etabonate only data in dog plasma are available. The plasma protein binding was reported to be greater than 90% [83].

Clearance

Clearance is a measure of drug elimination from the body. It is expressed as the volume of plasma or any other fluid that is completely cleared from the drug per time unit (L/h). For most drugs showing linear protein binding and non-saturated elimination this value is a constant. If the drug is mainly metabolized and cleared by the liver, the maximal clearance a drug can reach is the rate of the liver blood flow (~90 L/h). A rapid clearance after the drug enters the systemic circulation results in low systemic concentrations, minimizing the potential for systemic side effects and improving its therapeutic index.



* in dogs

Figure 2-3. Protein binding of inhaled and intranasal corticosteroids

All currently available corticosteroids for inhalation and intranasal use are cleared in the liver with values close to the liver blood flow. The clearance of such high extraction drugs is independent of protein binding. Therefore, further efforts to develop new steroids with increased intrinsic hepatic clearance is unnecessary, as such steroids will not be cleared more efficiently.

Research should rather focus on new drugs with extrahepatic elimination as this will be the only way to further increase the clearance over the liver blood flow [114]. Budesonide has the highest clearance rate with 84 L/h [81], followed by fluticasone propionate (66-90 L/h) [73, 74], flunisolide (57 L/h) [86], mometasone fuorate (54 L/h) [63] and triamcinolone acetonide (37 L/h) [84]. For the active metabolites 17-BMP and des-CIC, somewhat higher clearance rates (120 L/h and

228 L/h, respectively) have been reported [84]. However, these values are calculated based on the assumption of complete conversion of the prodrug. The precise determination of their clearance values is only possible after intravenous administration of these metabolites. The clearance of loteprednol etabonate was studied in dogs and found to be 0.9 L/h/kg, which is within the range of the other steroids [83]. Up to now, no human data is available. Table 2-1 shows the clearance values for the different corticosteroids.

Volume of distribution

The volume of distribution is a measure of the distribution of the drug in the body. It relates the plasma concentration to the amount of the drug in the body. The lower the concentration in the plasma, the more of the drug is distributed into the tissue, resulting in a larger volume of distribution. Thus, corticosteroids with a very large volume of distribution (300-900 L) are extensively distributed and bound to the tissues. However, there is not necessarily a direct correlation between the volume of distribution of a corticosteroid and its pharmacological activity. The pharmacological activity depends also on the concentration of unbound drug at the site of action and its receptor binding affinity. At steady-state, the unbound, free drug depends only on the clearance and the degree of protein binding, but not on the volume of distribution [62, 80]. Furthermore, when comparing volume of distributions, it has to be kept in mind that the values can differ depending on the way of calculation.

The volume of distribution at steady-state for the currently used corticosteroids is highest for FP (318-859 L) [62, 73, 115, 116] as well as for the active metabolites des-CIC (897 L) and BMP (424 L) [76]. Similar to the clearance, the values of the volumes of distribution of des-CIC and BMP are based on the assumption of complete

conversion from the prodrug to the active metabolite. Other volumes of distribution are 183-301 L for BUD [62, 75, 81, 115], 103 L for triamcinolone acetonide [62, 84, 115] and 96 L for flunisolide [62, 87]. Reported values for the different corticosteroids are also listed in Table 2-1. The volumes of distribution of the inhaled corticosteroids are in accordance with the lipophilicity of those drugs. The more lipophilic the drug is, the more is distributed into and bound to the tissues.

Half-life

The half-life is the time needed for the total amount of drug in the body or the concentration of the drug in plasma to decrease by one half its value. In inhalation therapy two different half-lives can be distinguished, the elimination half-life and the terminal half-life after inhalation.

The elimination half-life is dependent on the clearance and the volume of distribution. The elimination half-life is best determined after intravenous administration. It should be remembered that the half-life is dependent on both, the clearance and the volume of distribution. A large volume of distribution results in a long elimination half-life as can be seen for fluticasone propionate ($t_{1/2}$ = 7-8 h) [62, 72, 73, 80, 115]. Mometasone fuorate also has a long elimination half-life after intravenous administration. It is reported to be 5.8 h [70]. The other corticosteroids have shorter elimination half-lives reported as 2.8 h for budesonide [81, 97, 115] and 2.0 h for triamcinolone acetonide [62, 80, 81, 84, 97, 115] and 1.3 h for flunisolide [62, 80, 82, 86, 97, 115]. Daley-Yates et al. found a half-life of 0.5 h and 2.7 h after intravenous administration for the prodrug beclomethasone dipropionate and its active metabolite 17-beclomethasone monopropionate, respectively [76]. Ciclesonide and des-ciclesonide have a reported half-life of 0.36 and 3.4 h, respectively [88].

It is necessary to distinguish between the elimination half-life and the terminal half-life after inhalation as these can differ. For example, the half-life for fluticasone propionate is between 7-8 h after intravenous administration but increases to around 14 h after inhalation of the drug [68, 72, 116]. In the latter case the half-life is no longer determined by clearance and volume of distribution and therefore, by the elimination but rather by the absorption. Hence, the slower the terminal elimination half-life, the slower the drug is absorbed and the longer it is retained in the lungs [68, 97]. However, Thorsson et al. [116] reported the elimination half-life after intravenous administration of fluticasone propionate to be 14.4 h. They explain the long elimination half-life with an intensive distribution of the drug into the tissue.
Triamcinolone acetonide and 17-beclomethasone monopropionate show significantly longer terminal half-lives after inhalation than after intravenous administration [68, 77, 84, 97]. The longer terminal half-life after inhalation is positively correlated with the pulmonary residence time of the drug in the lung, therefore, increasing the efficacy of the drug. The other corticosteroids, such as beclomethasone dipropionate, budesonide, ciclesonide and flunisolide have terminal half-life values similar to the elimination half-life [62, 77, 80-82, 86, 97]. Table 2-1 summarizes the elimination and terminal half-lives for the different corticosteroids.

Hermann et al. [117] studied the pharmacokinetics and pharmacodynamics of intranasal administered loteprednol etabonate and fluticasone propionate. They found that the terminal half-lives of loteprednol and fluticasone after intranasal administration are around 2 h and around 4 h, respectively [116].

Mean absorption time (MAT)

The mean absorption time describes the average time it takes for a molecule of a drug to get absorbed into the systemic circulation [115]. This parameter can be used to estimate the duration of pulmonary retention for inhaled corticosteroids. A longer retention in the lung leads to a longer availability of the drug in the lung and hence, increases the release time, which is positively correlated with an increased local activity. Therefore, a longer MAT indicates a greater pulmonary residence [115]. The MAT's for fluticasone propionate, triamcinolone acetonide and budesonide are reported to be 5-7 h [118], 2.9 h [84] and around 1 h [118], respectively. The long MAT of fluticasone is in agreement with its relatively low aqueous solubility and might suggest a longer availability of fluticasone in the lungs [118].

Lipid conjugation

Another way of drug targeting to the lung/nose is the *in vivo* formation of lipid conjugates. Corticosteroids with a hydroxyl group in the 21-position are able to reversibly bind to fatty acids in the lung and nose, respectively. These lipid conjugates are not absorbed from the lung/nose into the systemic circulation and are not active [119]. Moreover, they retain the corticosteroid in the tissue, hence, acting as slow release reservoirs. From this depot the drug is gradually released by hydrolysis of the ester bond [68]. It should be kept in mind that lipid conjugation and lipophilicity are not the same. Lipid conjugation is a true chemical reaction, producing an ester bond between the corticosteroid and a fatty acid, whereas lipophilicity is a physicochemical characteristic of the compound itself [68]. It could be shown in several studies that budesonide is able to form those esters with fatty acids with the help of ATP and acetyl CoA (Figure 2-4) [119-123]. Compared to fluticasone propionate, budesonide is 6 to 8 times less lipophilic

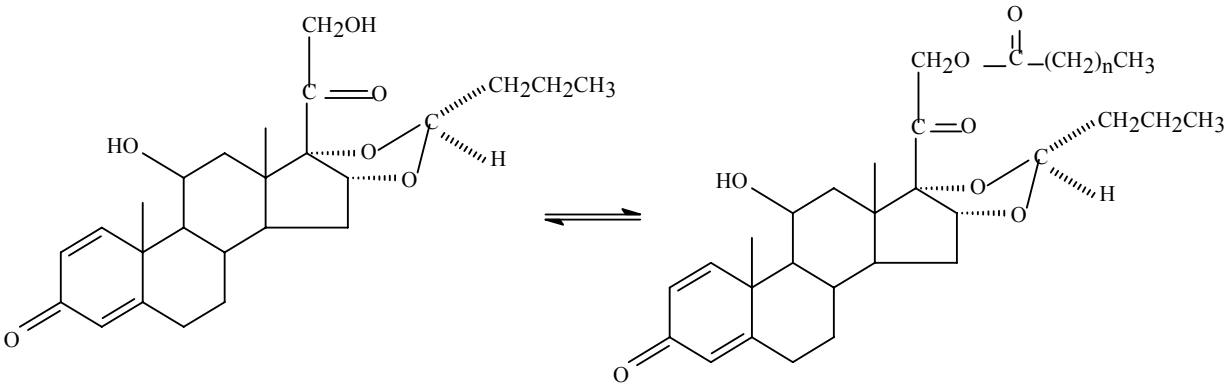
and its receptor binding affinity is also smaller than that of fluticasone propionate [124]. However, budesonide seems to be retained in the lung/nose for a longer time due to the highly lipophilic fatty acid esters, which increase the lipophilicity of budesonide 500 to 10,000 times [119, 122, 124]. The conjugation of budesonide with fatty acids is rapidly formed in airway and lung tissue. Only 20 min. after inhalative administration around 80% of budesonide retained in the large airways was found conjugated. No fatty acid conjugates were detected for fluticasone propionate, which is in accordance with its chemical structure [119].

The conjugation process is reversible. In several experiments, it could be shown that budesonide conjugates gradually hydrolyze and release free budesonide [119, 121, 122]. It seems that budesonide is esterified in most tissues to varying degrees. It could be found that the same fatty acid formation also happens in the nasal mucosa but not in peripheral tissue, prolonging the effect of budesonide in the treatment of allergic rhinitis without increasing the systemic side effects [122].

Des-ciclesonide, the active metabolite of the new corticosteroid ciclesonide, on the other hand has the necessary 21-hydroxyl group and, therefore is able to form fatty acid esters. This will lead to a longer retention time in the lungs allowing for a longer efficacy. The formation of lipid conjugates has not been related to adverse effects [88].

Although 17-beclomethasone monopropionate, flunisolide, and triamcinolone acetonide have the necessary free 21-hydroxyl group, these corticosteroids have not been shown esterification with fatty acids. The reason for that is not clear but it could be postulated that the groups at the 16- and 17-position sterically hinder the formation of the lipid conjugate.

The ideal corticosteroid will probably never exist but understanding these issues will guide in finding a corticosteroid that combines most of the desired characteristics. To be highly effective in the lung or nose, a corticosteroid should have a long pulmonary and nasal retention time, respectively.



Budesonide Budesonide-lipid conjugate

Figure 2-4. Lipid-conjugate formation of budesonide [Tunek, A., K. Sjoedin, and G. Hallstroem, *Reversible Formation of Fatty Acid Esters of Budesonide, an Antiasthma Glucocorticoid, in Human Lung and Liver Microsomes.* Drug Metab Dispos, 1997. **25**(11): p. 1311-1317]

This can be achieved by either high lipophilicity due to a slow dissolution of the drug in the lung and nose (fluticasone propionate) or by the way of lipid conjugation (budesonide, ciclesonide). Once the drug is absorbed into the systemic circulation it should be highly bound to plasma proteins and be cleared rapidly by the liver, decreasing the possibility of interaction with systemic glucocorticoid receptors. Additionally, it should have a low oral bioavailability and hence, limit the systemic availability of swallowed drug. A prodrug or soft drug structure is also beneficial in increasing the local effects and decreasing the unwanted side effects. Administration of the drug directly to the site of action is another important factor for the inhalative therapy. It is dependent on

the device used for drug delivery and its formulation. Much progress has been made over the last years to optimize corticosteroid therapy but there is still room for further improvement.

CHAPTER 3
PHARMACOKINETICS OF METHYLPREDNISOLONE IN MOTORCYCLE ACCIDENT VICTIMS WITH ACUTE SPINAL CORD INJURY

Introduction

Glucocorticoids have been extensively used in the treatment of acute spinal cord injury although their usefulness has been discussed controversially [29]. The majority of spinal cord injuries (SCI) resulting in the permanent loss of sensory and motor functions do not involve physical transection of the spinal cord, but rather contusion or compression [29]. This incident is followed by the cessation of impulse generation and conduction during the first few hours after injury and a decrease of the blood flow to the injured segment. Following the National Acute Spinal Cord Injury study 2 and 3 (NASCIS 2 and 3), glucocorticoids received particular attention in the treatment of acute spinal cord injury [23]. Methylprednisolone is the only corticosteroid that is extensively studied [125]. Because of its limited solubility, phosphate or hemisuccinate salt of MP is used in the therapy. A loading dose of 30 mg/kg methylprednisolone hemisuccinate (MPHS) followed by a constant rate infusion of 5.4 mg/kg/h over 24 h has been used in SCI [24, 126, 127]. Pharmacokinetics of the phosphate salt (MPP) and the hemisuccinate salt (MPHS) in healthy male subjects have been reported earlier [128-130]. Plasma pharmacokinetic profile of MP in patients with SCI after administering MPHS has been recently reported [127]. Studies in pigs [131] have shown that after IV bolus dose of 30 mg/kg MPHS followed by 5.4 mg/kg/h infusion for 4 h, the cerebro-spinal fluid (CSF) levels of MP accounted for only 1.5% of the total plasma levels. It was experimentally

shown that p-glycoprotein efflux pumps present in the blood brain barrier (BBB) are responsible for keeping the drug out of CSF. The relationship between the plasma and the CSF levels of MP in humans has not been elucidated in detail.

However, there is still a controversy about the beneficial effects of methylprednisolone in the treatment of ASCI. Short et al. [132] concluded from their review that high dose methylprednisolone does not improve neurological recovery. Hugenholtz [38] comes to a similar conclusion, although he suggests that methylprednisolone is still a treatment option although not a standard of care. So far, this controversy is still going on and a final decision on the positive or negative effects has not been made.

Materials and Methods

Study Design and Sampling

Our study was carried out at the University of Bochum in Germany after approval by the local ethics committee. The patients were motorcycle accident victims with spinal cord injury. The study population consisted of 26 patients (19 male; 7 female) with a mean age of 45 ± 16 years (range: 15-81 years) and a mean weight of 72 ± 11 kg (range: 42-83 kg). Excluded were patients with life-threatening morbidity, pregnant women, patients undergoing previous corticosteroid therapy and drug addicts. Within 0.5-2 h after the accident, MPHS was administered as an IV bolus loading dose (30 mg/kg, over 15 min) and an IV infusion (5.4 mg/kg/h), which started at 1 h after starting IV dose and continued until 24 h (23 h infusion). Blood, CSF and saliva samples were collected frequently but not necessarily at prespecified time intervals up to 48 h. Plasma was immediately separated and all the samples were stored at –20°C until analysis. A total of 486 data points were available for the analysis.

Chemicals

MP, MPHS, and triamcinolone acetonide were obtained from Fisher Scientific. Ammonium sulfate and all other analytical grade chemicals were obtained from Fisher Scientific or equivalent sources.

Preparation of Standard Solutions

Primary stock solutions were prepared by dissolving methylprednisolone hemisuccinate in methanol to obtain a concentration of 1 mg/mL. Further stock solutions were prepared in methanol. The calibration curve was prepared by adding known amounts of methylprednisolone hemisuccinate to pooled human plasma to obtain concentrations ranging from 0.5 – 10 µg/mL. The quality controls were prepared from a second set of stock solutions which were added to pooled human plasma to obtain concentrations of 1 µg/mL, 5 µg/mL and 10 µg/mL.

Sample Extraction

Concentrations of MPHS and MP were measured by an HPLC method that was established by Rohdewald et al. [133]. Briefly, plasma samples (0.5 mL of blank, spiked or the patients' sample) were mixed with 0.1 mL of internal standard (triamcinolone acetonide, 20 µg/mL) and 1 g of ammonium sulfate. The samples were extracted with 2x3 mL of ethyl acetate. The organic phase was separated by centrifugation at 3000 rpm for 10 min. The organic phase was evaporated to dryness over a stream of nitrogen gas. The residue was reconstituted in 0.1 mL of mobile phase, consisting of acetonitrile : water : glacial acetic acid (70:30:2, v/v/v), and analyzed by HPLC.

HPLC Conditions

The HPLC consisted of an LDC/Milton Roy pump (Model IIIG), a 20 µL sample loop, a C-18 column (Zorbax 15 cm x 4.6 mm ID, Hewlett Packard), an UV detector at 254 nm (LDC Analytical), and a Hewlett Packard HP 3396 Series III Integrator. The mobile phase consisted of acetonitrile:water:glacial acetic acid (70:30:2, v/v/v) and was pumped at 1.5 mL/min. Calibration curves in human plasma were drawn in the concentration range of 0.5-10 µg/mL. Quality control samples were used at 1, 5, and 10 µg/mL during the analysis of the patients' samples. The calibration curves were linear over the 0.5-10 µg/mL range ($r>0.95$).

Data Analysis

The pharmacokinetics of MPHS and MP after the administration of MPHS by IV bolus and IV infusion were assessed by population pharmacokinetic analysis using NONMEM (nonlinear mixed effect modeling) software (Version V). The codes were written in ADVAN 9 (TRAN 1) as a set of differential equations and a first order conditional estimation (FOCE) method was used for analysis. Plasma concentrations of MPHS and MP, and CSF concentrations of MP (total of 486 data points) were used for the population modeling. Different models assessed inter-subject and intra-subject variability parameters. The diagnostic parameters included minimum objective function (OBJF), distribution of the residuals, magnitude of the fixed and random effect parameters, correlation between the observed and the population predicted concentrations, and correlation between the observed and the individual predicted concentrations.

Results and Discussion

Preliminary MPHS was rapidly converted into MP as seen in the initial high levels of MP after administering the prodrug. However, measurable MPHS levels could be observed even 24 h after the end of the infusion. MP was rapidly distributed into the peripheral compartment, which was reflected in its rapid appearance in CSF. Mean steady-state levels of MPHS were 17.6 ± 11.0 μg/mL and 2.76 ± 2.61 μg/mL in plasma and CSF, respectively. At steady-state, the average measured MP concentration in plasma was 12.3 ± 7.0 μg/mL and 1.74 ± 0.85 μg/mL in CSF. MPHS levels in CSF showed high variability. MPHS was not detected in saliva while the average measured salivary levels of MP were available for only 6 patients and accounted for 32% of the respective plasma levels (mean ratio of 0.32, range 0.01-1.09). Therefore, only plasma and CSF levels of MP and plasma levels of MPHS were considered for the analysis.

Differential equations were set up with the prior assumption of two-compartment models for both MPHS and MP (Figure 3-1) [129, 130].

Concentrations of MP in CSF were calculated by assuming CSF as a part of the peripheral compartment. The model was simplified using the following assumptions that MPHS is completely converted into MP; all the rate processes are of first order; CSF is a part of the peripheral compartment and the drug concentration in CSF equals the free drug concentration in plasma; volume of CSF is 125 mL [134]; and plasma protein binding of MP is 78% and binding does not occur in CSF [130].

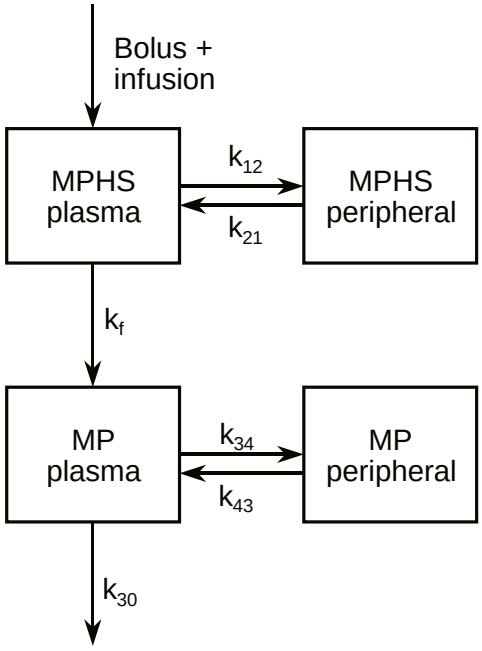


Figure 3-1. Pharmacokinetic model for methylprednisolone hemisuccinate (MPHS) and methylprednisolone (MP) after administration of MPHS

The pharmacokinetics of MPHS and MP were adequately described by the present model. The inter-subject variability in the pharmacokinetic parameters and the residual error model were best described by exponential distribution models. The population pharmacokinetic model parameters are summarized in Table 3-1.

The mean pharmacokinetic profiles of the MPHS and MP in plasma and CSF along with the observed data points are summarized in Figure 3-2, which reflects the applicability of the model.

Table 3-1. Population estimates of the PK parameters for MPHS and MP after high doses in patients with acute spinal cord injury.

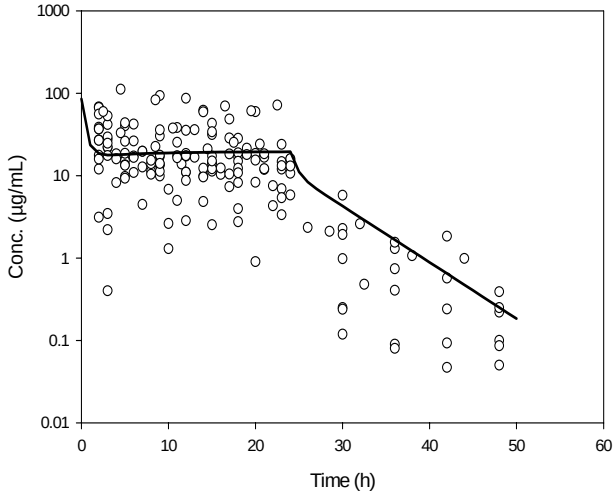
Parameter	Mean	CV (%)
V_{MPHS} (L)	25.6	59.0
k_f (h^{-1})	0.783	29.8
k_{12} (h^{-1})	0.652	23.7
k_{21} (h^{-1})	0.321	28.4
V_{MP} (L)	21.1	89.3
k_{30} (h^{-1})	1.87	96.9
k_{34} (h^{-1})	0.0019	87.1
k_{43} (h^{-1})	0.415	111.4
Residual variability		
σ (MPHS-plasma)	89.6%	
σ (MP-plasma)	48.3%	
σ (MP-CSF)	70.4%	

Representative concentration-time profiles in four subjects for MPHS and MP in plasma and CSF are given in Figure 3-3. The salivary levels of MP were compared with the CSF profile of MP and lack of agreement between the salivary and the CSF levels of MP is represented in Figure 3-4. The population mean estimates of the clearance for MPHS and MP were 20.0 L/h and 39.5 L/h, respectively.

Validation

Two six point calibration curves ranging from 0.5 to 10 µg/mL of MPHS and MP were run each day on three separate days. The calibration curves were linear over the entire concentration range (r^2>0.98). Using linear least square regression, plots were generated, showing the peak-area ratios of the analyte to the internal standard (I.S.) versus the nominal concentration of the calibration standards (Figure 3-5 and 3-6).

A



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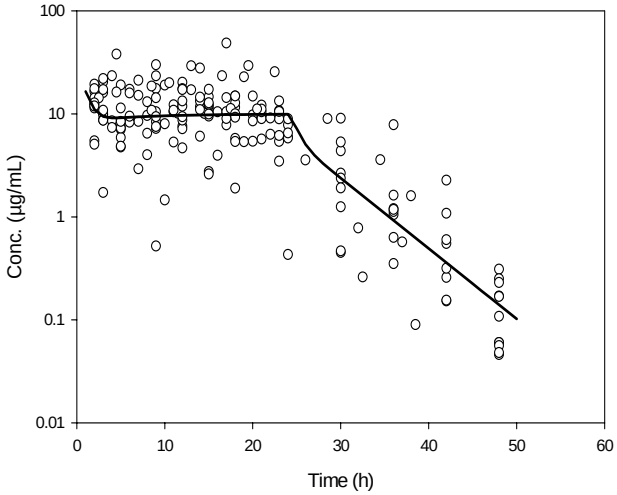

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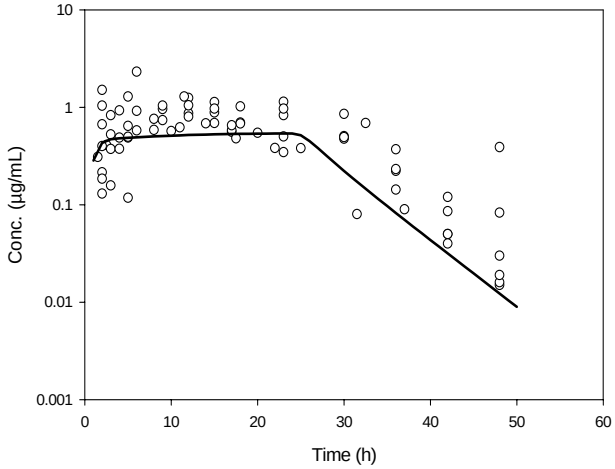

Figure 3-2. Observed concentrations and the predicted profile using the population mean pharmacokinetic parameters for MPHS and MP in plasma (A and B) and MP in CSF (C) based on a mean body weight of 73 kg.

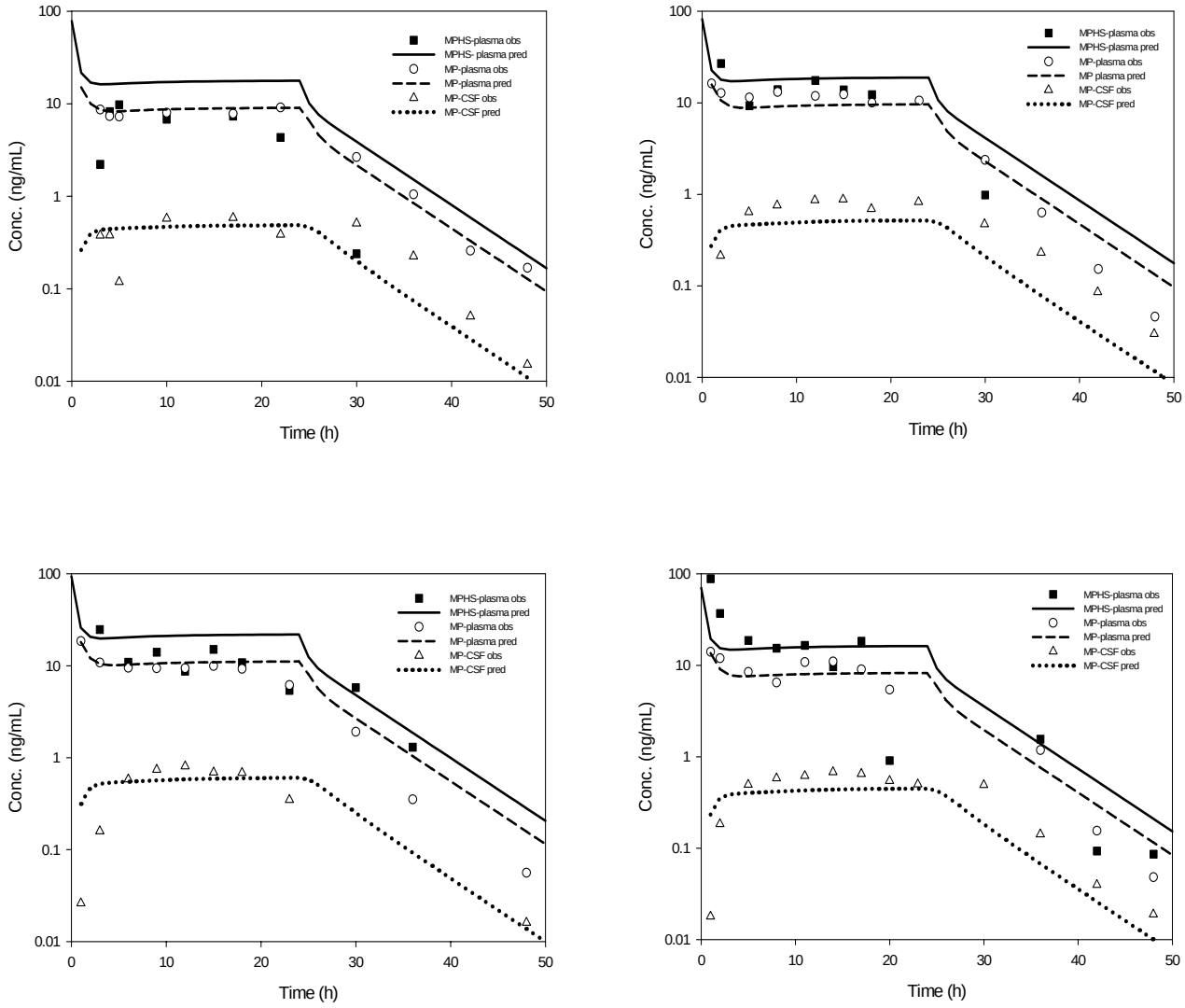

Figure 3-3. Pharmacokinetic profiles for MPHS and MP using Bayesian parameter estimates in four representative patients.

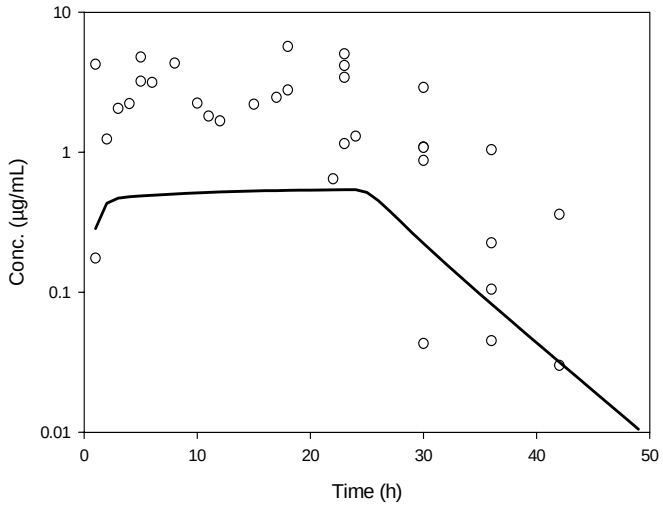

Figure 3-4. Relationship between the observed salivary levels of MP and the predicted profile of MP in CSF

The calibration curves were used to calculate the concentration of independently prepared quality controls. The intra- and inter-batch precision and accuracy data for MP and MPHS are shown in Table 3-2 through 3-5.

High intravenous doses of methylprednisolone started within 8 h of acute SCI have been used in the clinic since the conclusion of the NASCIS 2 and this dosing regimen has been confirmed by NASCIS 3 [126].

Glucocorticoids, given at high doses have shown to facilitate the excitability of the remaining spinal neurons, enhance the regional blood flow and reduce spinal cord lipid peroxidation and tissue degeneration [16, 24, 29, 126, 135]. Further, NASCIS 3 has concluded that if the MP treatment is started within 3 h after the injury, the optimum duration of treatment should be 24 h and if initiated between 3 and 8 h after injury, the maintenance dose infusion should be extended up to 48 h [126].

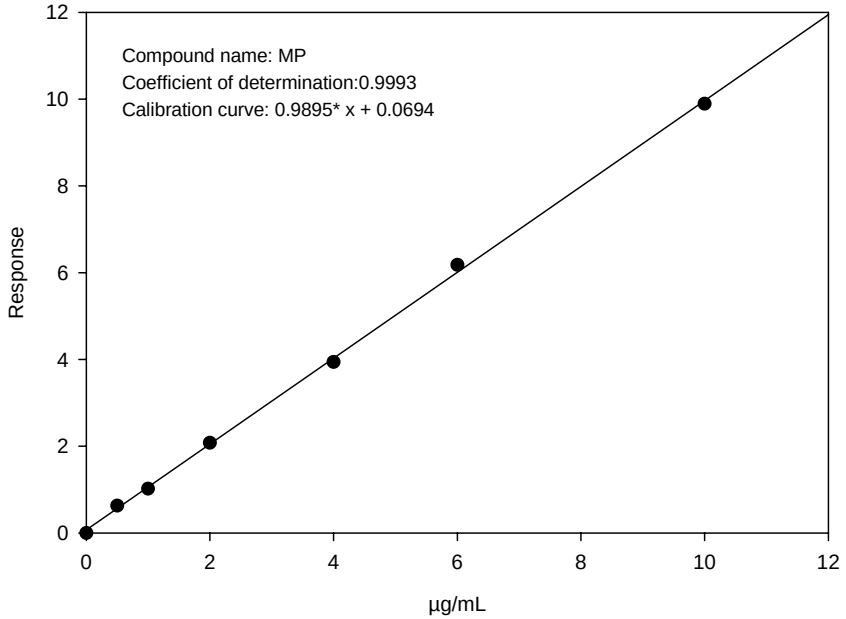

Figure 3-5. MP—Representative calibration curve in plasma

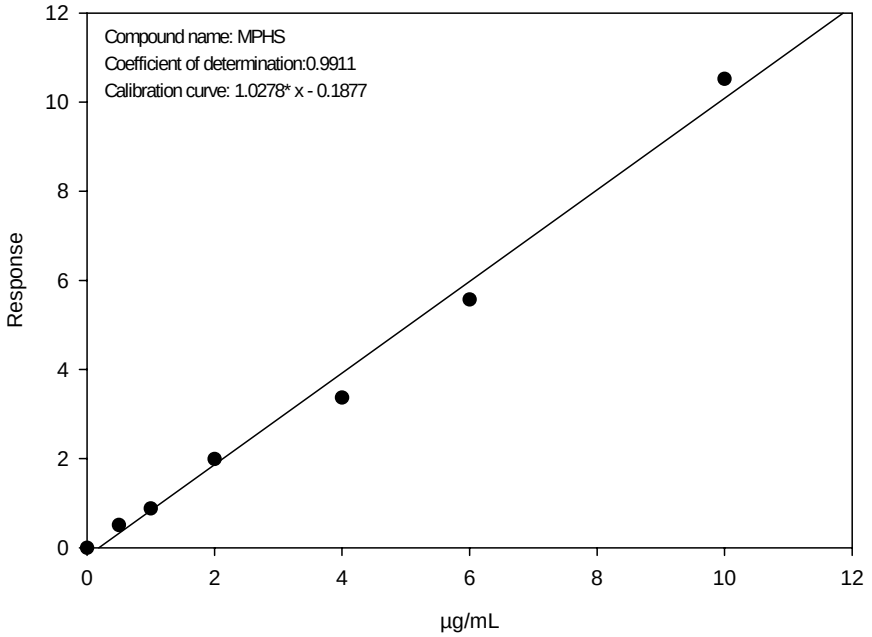

Figure 3-6. MPHS—Representative calibration curve in plasma

Table 3-2. MP standard concentrations—Inter-day precision and accuracy

MP µg/mL*						
Theoretical Concentration	0.5	1	2	4	6	10
Mean	0.58	0.90	2.09	3.97	5.88	10.08
SD	0.09	0.19	0.16	0.26	0.34	0.31
% Bias	16.67	-10.17	4.42	-0.79	-2.06	0.75
% CV	15.77	21.69	7.50	6.64	5.76	3.08

*n= 6

Table 3-3. MPHS standard concentrations—Inter-day precision and accuracy

MPHS µg/mL*						
Theoretical Concentration	0.5	1	2	4	6	10
Mean	0.53	0.98	2.14	3.98	5.87	10.06
SD	0.19	0.24	0.13	0.35	0.34	0.36
% Bias	6.67	-2.5	6.92	-0.42	-2.14	0.57
% CV	35.47	24.89	6.09	8.87	5.71	3.62

*n= 6

Table 3-4. MP quality controls—Inter- and intra-batch precision and accuracy

Theoretical Concentration	1	5	10
MP Batch 1 (n=4)			
Mean	1.27	4.80	9.72
SD	0.64	0.16	0.52
% bias	26.5	-3.95	-2.85
% CV	50.43	3.35	5.33
MP Batch 1 (n=4)			
Mean	1.41	4.86	9.65
SD	0.72	0.18	0.26
% bias	41.25	-2.75	-3.48
% CV	51.16	3.65	2.65
MP Batch 1 (n=4)			
Mean	1.30	4.91	9.86
SD	0.57	0.21	0.23
% bias	29.75	-1.85	-1.40
% CV	43.90	4.27	2.38

Table 3-5. MPHS quality controls—Inter- and intra-batch precision and accuracy

Theoretical Concentration	1	5	10
MPHS Batch 1 (n=4)			
Mean	1.11	4.74	9.60
SD	0.1	0.07	0.54
% bias	10.75	-5.25	-4.03
% CV	9.01	1.45	5.58
MPHS Batch 1 (n=4)			
Mean	1.15	4.75	9.37
SD	0.13	0.30	0.45
% bias	15	-5.05	-6.23
% CV	10.88	6.35	4.83
MPHS Batch 1 (n=4)			
Mean	1.18	5.51	10.90
SD	0.28	0.30	0.31
% bias	18.25	10.25	9.03
% CV	23.55	5.37	2.83

Information on the pharmacokinetics of both MPHS and MP in plasma and the extent of their levels in CSF are scarce in patients with ASCI. Segal et al [127] have reported the pharmacokinetics of MP in plasma of these patients following the administration of MPHS according to NASCIS recommendation (30 mg/kg IV bolus followed by a 5.4 mg/kg/h continuous infusion up to 24 h). Standard two-stage analysis was used to fit the data to an one-compartment model. The authors found an inverse correlation between the clearance estimate (mean: 30 L/h) and the spinal cord injury score. In our study, the pharmacokinetics of MPHS and MP were determined in patients with ASCI who were treated with MPHS according to NASCIS recommendations. The treatment was initiated within 0.5-2 h after the accident. Large variations in corticosteroid plasma and CSF concentrations were observed despite weight-adjusted dose and this variability was more pronounced for MPHS. This might be due to the fact that the patients represented emergency cases of SCI and hence, additional therapeutic treatment such as intravenous blood substitution was often necessary. In our study, the plasma

levels of MPHS and plasma and CSF levels of MP were considered for the pharmacokinetic analysis. The sampling was sparse in some patients and hence a population analysis was carried out to describe the pharmacokinetics of MPHS and MP.

The terminal phase half-life estimate of MPHS was 4.4 h, and intact prodrug was still detectable in plasma 24 h after cessation of the infusion. This observation is in contrast to the short half-lives that were reported in healthy subjects (less than 1 h, even at high doses) [128, 129, 136]. This difference might be due to the pathophysiological changes, such as hypoperfusion, associated with the accident-related shock and ASCI. Furthermore, the clearance estimate for MPHS in the present study (20.0 L/h) was much lower than that in previous reports. The average clearance estimates for MPHS at single IV doses of 250 mg and 1000 mg to healthy subjects were 40.1 ± 13.4 and 42.3 ± 11.4 L/h, respectively [128]. A reduction in MPHS clearance (15.6 L/h) has been reported in patients during cardiopulmonary bypass surgery with a mean terminal half-life of 0.92 h [137]. On entering the systemic circulation, MPHS is hydrolyzed rapidly into MP by carboxylesterase enzymes present in the blood, the liver, and kidneys [138]. Blood flow to these organs is decreased during shock conditions and this might be responsible for slower elimination of MPHS.

Pharmacokinetics of MP after the administration of MPHS have been reported in healthy volunteers [128, 129, 136, 139] and in the patients [127, 140-144]. The mean estimate of clearance of methylprednisolone in our study was 39.5 L/h and was comparable to 30 L/h reported previously in patients with ASCI [127]. The clearance estimates of MP after multiple high doses of MPHS were higher than after single and low doses of MPHS. This observation was attributed to dose-dependent prodrug hydrolysis

[136]. The appearance of MP in CSF following an IV dose has been reported to be rapid in cats and pigs and reached C_{max} within 5 min [131, 145]. However, a slow equilibrium between the plasma and CSF levels of MP has been described in patients with multiple sclerosis wherein the plasma to CSF concentration ratio decreased from 150 at 1 h to 15 at 6 h [144]. In our study, the CSF levels of MP rose quickly and the plasma to CSF ratio was fairly constant throughout the infusion period.

The CSF concentrations of MPHS and MP were ~16% and 14% of the respective plasma concentrations even though the MPHS levels in the CSF were highly variable. This CSF-plasma ratio for MP is in contrast to that in patients with multiple sclerosis where CSF accounted for about 7% of the plasma concentrations of MP [144]. P-glycoprotein efflux pumps are present on the brain capillary endothelial cells and form an important part of the blood-brain barrier [146, 147]. Glucocorticoids are known substrates for p-glycoprotein [148] and the absorption of MP from the rat small intestine is shown to be restricted by p-glycoprotein [149]. Studies in pigs [131] have shown that after IV bolus dose of 30 mg/kg followed by 5.4 mg/kg/h infusion for 4 h (MPHS), the CSF levels of MP accounted for only 1.5% of the plasma levels. The same study, using wild type mice and transgenic mice that lack two key p-glycoprotein genes (*mdr 1a/1b* knockout mice), showed that p-glycoprotein is involved in keeping MP out of the spinal cord. These results were in contrast with another study in guinea pigs where the CSF accounted for 21% of the total plasma levels of MP after high IV bolus dose of MP (20 mg/kg) [150]. In our study, the CSF levels of MP were adequately explained by the PK model consisting of CSF as a part of peripheral compartment. Species differences in

the pharmacokinetics and/or health status of the subjects might be the reason for these varying ratios of plasma to CSF concentrations of MP.

Analysis of saliva showed that MPHS is not secreted into saliva, similar to previous observation [128]. MP could be monitored in saliva and it accounted for 32% of the respective plasma levels. However, the saliva concentrations of MP were higher than the corresponding CSF concentrations (Figure 3-4). The observed saliva levels were higher than those previously measured in healthy volunteers where the levels were comparable to the unbound concentrations in plasma [128]. It should be mentioned that in our study saliva levels were only available in six patients. However, saliva monitoring may be a simple approach of drug level monitoring as a surrogate for expected CSF concentrations.

Our study shows clearly that SCI treatment with the employed dosing regimen results in MP plasma and CSF steady-state concentrations within the µg-range (10^{-5} M), which is far above the receptor saturation range [130, 151]. MP plasma concentrations were above 10^{-5} M for more than 24 h. About 10-40% of the corresponding plasma levels were observed in the CSF, indicating that only the free, non-protein bound fraction of MP (approximately 22%) enters CSF. Hence, the concentrations are sufficiently high for the induction of non-receptor mediated, unspecific membrane effects.

CHAPTER 4
A COMPUTER ALGORITHM FOR THE ASSESSMENT OF THE DOSE-DEPENDENT PHARMACOKINETICS AND PHARMACODYNAMICS OF PREDNISOLONE BASED ON LINEAR AND NONLINEAR PROTEIN BINDING

Introduction

Corticosteroids are very valuable agents in the treatment of inflammatory diseases. Despite their beneficial effects, they also exhibit numerous unwanted systemic side effects. The major side effects include lymphopenia, hyperglycemia, osteoporosis, changes in bone mineral density, growth retardation in children, cataracts, glaucoma, skin thinning and suppression of the hypothalamus-pituitary-adrenal (HPA) axis due to a negative feedback mechanism [3]. These side effects have been known for a long time and improving the benefit-to-risk ratio has been a topic of investigations over a number of years.

Most of the synthetic corticosteroids are moderately to highly protein bound (≥70%). Since it is understood that only the free, unbound drug is pharmacologically active, knowledge about the protein binding might be important in assessing the pharmacokinetics and pharmacodynamics of a drug. Currently, dosing regimens are based on total drug concentrations rather than free plasma concentrations. In case of linear protein binding the plasma concentration of the free drug is a constant fraction of the total drug and concentration-time profiles are easy simulated. However, if nonlinear protein binding occurs this issue becomes more difficult because the fraction of the unbound drug is not constant and an increase in the free plasma concentration can have a significant effect on the outcome of drug therapy as this increase will not be accounted

for in a dosing regimen based on the total drug concentration [53]. The relationship between the total and the free drug concentration for linear and nonlinear drugs is shown in Figure 4-1. In linear kinetics the free drug concentration increases proportionally with increasing total concentration whereas in the case of nonlinear kinetics this relationship is unproportional leading to higher free concentrations.

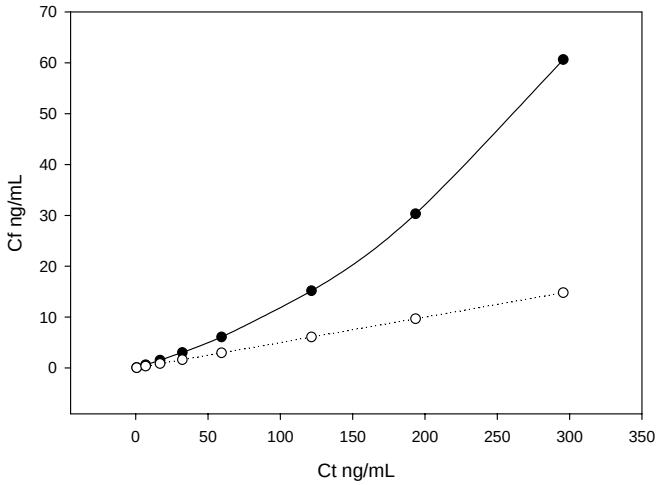

Figure 4-1. Total versus free plasma concentration for linear and nonlinear drugs

Prednisolone and its esters are commonly prescribed drugs for oral and intravenous administration, respectively. Prednisolone has a higher potency and longer duration of action than hydrocortisone and its systemic availability of 82% is quite high and limited by first-pass liver metabolism rather than by incomplete absorption [152]. The disadvantage of prednisolone is its nonlinear pharmacokinetics in humans. The nonlinear binding characteristics have been studied by several groups [39, 43, 46, 152-154]. As with hydrocortisone, this nonlinearity is due to saturable nonlinear protein binding. Prednisolone binds to two proteins, albumin and human corticosteroid binding globulin (CBG, transcortin). Albumin, one of the most prevalent proteins in the human blood, has

a low affinity but high capacity for steroids while transcortin, an α1-acid-glycoprotein, has a high affinity but low capacity for binding glucocorticoids [43]. It is easily saturated when plasma concentrations of the exogenous corticosteroid are increased. With increasing doses the free fraction increases in a nonlinear way until transcortin is saturated and pharmacokinetic parameters based on the total drug will change with dose. At low concentrations prednisolone binds to both proteins transcortin and albumin, resulting in a constant free fraction. As concentrations approach and exceed the binding capacity of transcortin the free fraction increases nonlinearly. After saturation of transcortin the binding becomes linear as only linear binding to albumin occurs. Hence, it might be a better approach to use the free, unbound concentration of prednisolone to calculate pharmacokinetic parameters, to predict its concentration-time profile and effect and to evaluate the degree of systemic side effects by determining the suppression of endogenous cortisol. The nonlinear relationship between total prednisolone concentration and fraction bound to proteins is shown in Figure 4-2. The solid line shows a simulation of the fraction unbound of prednisolone over a wide concentration range while the dots represent actual data.

The suppression of endogenous cortisol after administration of a synthetic corticosteroid has been used as a surrogate marker to quantify the degree of systemic steroid activity and therefore also of its side effects [155]. The degree of cumulative cortisol suppression (CCS) after single or multiple doses is usually determined as the difference of the area under the plasma concentration-time curves (AUC) between the placebo and the given drug over a 24-hour period [156].

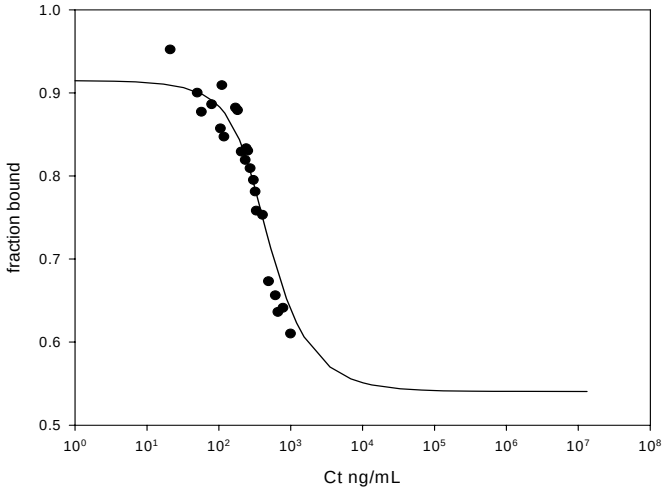

Figure 4-2. Relationship between the fraction bound and the total concentration of prednisolone.

It was the purpose of our study to predict the dose-dependent pharmacokinetics of prednisolone based on linear and nonlinear binding characteristics and link those to its pharmacodynamic effects using an indirect response model. Finally, an algorithm was developed to allow predictions and comparisons of the CCS of other corticosteroids with prednisolone.

Materials and Methods

The software program Microsoft Excel (Microsoft Excel 97 SR-2, Microsoft, Redmond, WA) was used for the pharmacokinetic simulations and the calculation of the CCS. Prednisolone was chosen as a reference for the simulations because it is prescribed frequently and has nonlinear protein binding. Furthermore, prednisolone has another unique characteristic in that it competes with endogenous cortisol for protein binding sites on transcortin. Such competition is of interest because it may alter the pharmacokinetics of prednisolone and cortisol through changes in the free fraction of these compounds in blood [43].

Plasma concentration-time data of free and total prednisolone and free and total prednisolone sodium phosphate after intravenous and oral administration were obtained from previous studies [44, 51]. Then, the pharmacokinetic parameters of free and total prednisolone plasma concentrations were determined by curve fitting using MicroMath Scientist for Windows™ software (Version 2.0, MicroMath Inc.) (Table 4-1, 4-2).

Total prednisolone sodium phosphate plasma concentration data after IV administration could be fitted to a two-compartment body model (Equation 4-1)

$$C_t = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} \tag{4-1}$$

After administration the ester rapidly hydrolyzes and releases the free alcohol. This process is believed to be almost instantaneous and complete [152].

A three-compartment and one-compartment body model could be used to fit the free prednisolone plasma concentrations after single intravenous (Equation 4-2) and oral administration (Equation 4-3) respectively.

$$C_f = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} + C \cdot e^{-\gamma \cdot t} \tag{4-2}$$

after IV administration, where A, B, C, α, β and γ = macroconstants and α, β and γ refer to the distribution phase, the rapid elimination and the slow elimination phase, respectively, and

$$C_f = \frac{F \cdot D \cdot k_a}{V_d \cdot (k_a - k_e)} \cdot \left(e^{-k_e \cdot t} - e^{-k_a \cdot t} \right) \tag{4-3}$$

after oral administration where F= bioavailability, D= dose, V_d= volume of distribution, k_a= first order absorption rate constant and k_e= first order elimination constant.

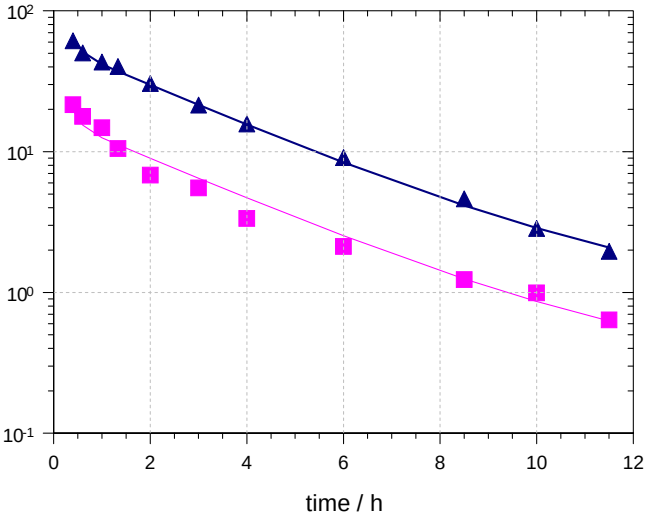

Figure 4-3. Free prednisolone plasma concentration after IV administration of 49.2 mg (Δ) and 16.4 mg (□)

Table 4-1. Pharmacokinetic parameter of free prednisolone after IV administration

Parameter	
alpha (h^{-1})	6.850
beta (h^{-1})	0.452
gamma (h^{-1})	0.100
k21 (h^{-1})	0.108
k31 (h^{-1})	1.485
Vc (L)	16.498

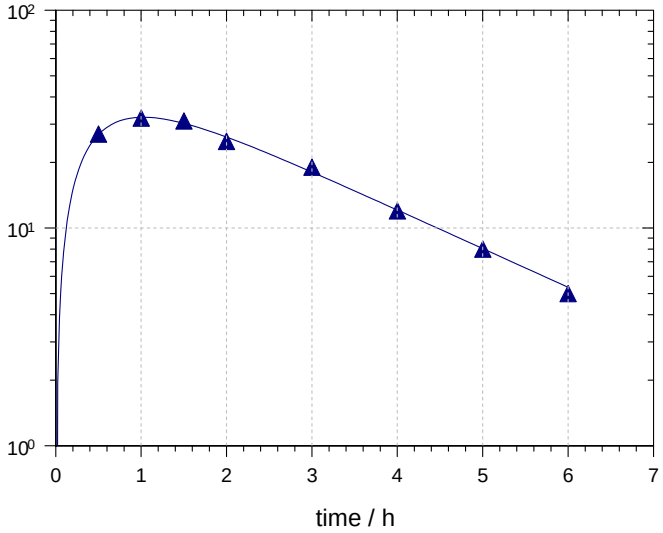

Figure 4-4. Free prednisolone concentration after oral administration of 14 mg

Table 4-2. Pharmacokinetic parameter of free prednisolone after oral administration

Parameter	
F (%)	82
k_a (h^{-1})	0.409
k_e (h^{-1})	1.916
V_c (L)	50.011

In case of multiple dosing Equations 4-4 and 4-5 have to be used for intravenous and oral administration, respectively.

$$C_f = A \cdot \frac{e^{-\alpha \cdot t}}{1 - e^{-\alpha \cdot \tau}} + B \cdot \frac{e^{-\beta \cdot t}}{1 - e^{-\beta \cdot \tau}} + C \cdot \frac{e^{-\gamma \cdot t}}{1 - e^{-\gamma \cdot \tau}} \tag{4-4}$$

$$C_f = \frac{D \cdot F \cdot k_a}{(k_a - k_e) \cdot Vd} \cdot \left(\frac{e^{-k_e \cdot t}}{1 - e^{-k_e \cdot \tau}} - \frac{e^{-k_a \cdot t}}{1 - e^{-k_a \cdot \tau}} \right) \tag{4-5}$$

The concentration dependence of nonlinear and linear plasma protein binding on the total drug was then determined by Equation 4-6

$$C_t = \frac{K_t \cdot P_t \cdot C_f}{1 + K_t \cdot C_f} + K_a \cdot P_a \cdot C_f + C_f \qquad (4\text{-}6)$$

where C_t = total plasma concentration, C_f = free plasma concentration, K = affinity constant, P = protein concentration and the subscripts t and a = transcortin and albumin, respectively. This equation describes the protein binding of prednisolone without competition for the binding sites on either transcortin or albumin (no cortisol present). If cortisol is present, cortisol and prednisolone compete for binding sites and Equation 4-6 must be modified to account for this competition [43, 53, 54]. Equation 4-7 represents the modification of Equation 4-6.

$$C_t = \frac{K_t \cdot P_t \cdot C_f}{1 + K_t \cdot C_f + K_t^C \cdot C_f^{Cort}} + K_a \cdot P_a \cdot C_f + C_f \qquad (4\text{-}7)$$

where K_t^C = affinity constant for cortisol to transcortin and C_f^{Cort} = free cortisol concentration. According to Rocci et al. [43] there is no competition between cortisol and prednisolone for albumin binding sites *in vivo* and therefore the binding of prednisolone to albumin is unaffected by the presence of cortisol.

Cumulative Cortisol Suppression

The derivation of the pharmacokinetic/pharmacodynamic approach, using an indirect response model, is described in detail elsewhere [157, 158]. Briefly, the circadian release of endogenous cortisol can be described by the following two linear equations. Between the acrophase t_{max} and t_{min} the release rate R_C (in concentration/time) decreases according to Equation 4-8

$$R_C = \frac{R_{max}}{V_d \cdot (t_{max} - t_{min} - 24)} \cdot t - \frac{R_{max} \cdot t_{min}}{V_d \cdot (t_{max} - t_{min} - 24)} \cdot t \qquad (4\text{-}8)$$

where R_C = daily cortisol release rate at baseline situation, R_{max} = maximum release rate (amount/time), V_d = volume of distribution, t_{max} = time of maximum release rate, t_{min} = time of minimum release and t = actual running time.

Between t_{min} and t_{max}, R_C increases according to Equation 4-9

$$R_C = \frac{R_{max}}{V_d \cdot (t_{max} - t_{min})} \cdot t - \frac{R_{max} \cdot t_{min}}{V_d \cdot (t_{max} - t_{min})} \cdot t \tag{4-9}$$

The resulting change in plasma cortisol concentration (C_{Cort}) under baseline conditions can then be calculated using Equation 4-10

$$\frac{dC_{Cort}}{dt} = R_C - k_e^{Cort} \cdot C_{Cort} \tag{4-10}$$

with k_e^{Cort} = first order elimination rate constant of cortisol and R_C as defined above [158]. Based on Equation 4-10, an indirect response model is used (Equation 4-11) to determine the change in cortisol plasma concentration after administration of an exogenous corticosteroid, relating the free corticosteroid concentration to the effect on cortisol release.

$$\frac{dC_{Cort}}{dt} = R_C \cdot \left(1 - \frac{E_{max} \cdot C_f}{EC_{50} + C_f}\right) - k_e^{Cort} \cdot C_{Cort} \tag{4-11}$$

where E_{max} = maximum suppressive effect, EC_{50} = free plasma concentration of the corticosteroid that produces 50% of the maximum suppressive effect and C_f = free plasma concentration of the exogenous corticosteroid.

Since the maximum possible effect is complete suppression of cortisol release, E_{max} is fixed to 1.

Results and Discussion

A total of four Excel spreadsheets were set up for intravenous and oral administration to quantify the pharmacokinetics and pharmacodynamics. Each route of administration requires two spreadsheets, one for single and one for steady-state multiple doses. In each spreadsheet the user has the option of using either compartmental or noncompartmental parameters and also decide between either a two- and three-compartment body model and one- and two-compartment body model for intravenous and oral administration, respectively. Additional to those options, there are required inputs for every situation. These include dose, dosing interval (for multiple dosing), time of dose, binding parameters and values for the hepatic blood flow and the EC_{50} of the free drug (E_{max} is fixed to 1, the maximum suppression).

In each spreadsheet the input and output parameters of a new drug can be compared to the reference parameters of prednisolone. As previously mentioned, it is always more difficult to make predictions for drugs showing nonlinear pharmacokinetics because the free drug fraction is not a constant as it is in linear pharmacokinetics. Therefore, the input parameters are based on the free plasma concentration of the drug except for the binding parameters, which are needed for the calculation of the CCS of the total drug. The output column shows the percentage of the total and free CCS (calculated as the percentage difference in the areas under the plasma cortisol concentration-time curves over 24 h between the placebo and the drug-treated groups), the terminal half-life of the free drug, its volumes of distribution depending on the compartments assumed, clearance, and the micro-constants. The value of the total CCS was added to show that in case of nonlinear protein binding, using the total plasma concentration would result in a higher degree of

systemic steroid activity and therefore in an overestimation of the effects and side effects of the exogenous corticosteroid. Figure 4-5 shows a screen capture of the user surface of the spreadsheet after single IV bolus administration and Figure 4-6 after multiple IV bolus administrations.

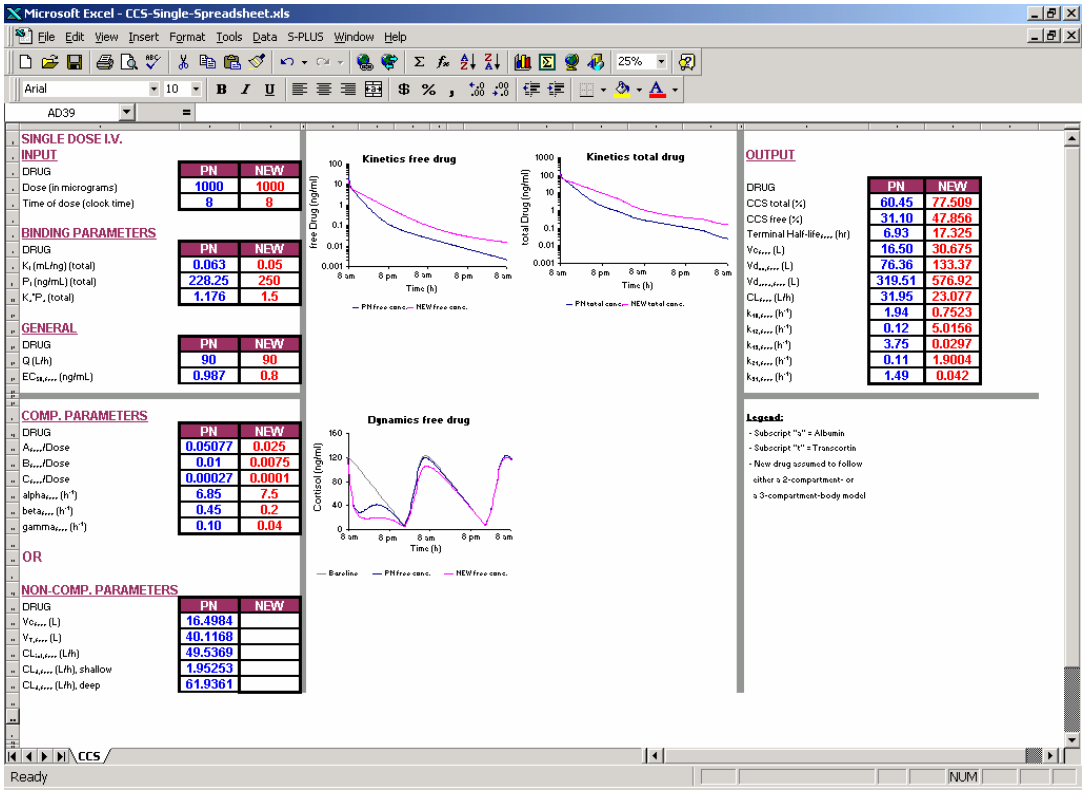

Figure 4-5. Screen capture of the user surface after single IV bolus administration

For the situation of single dose administration, the cortisol AUC is calculated up to 48 h after the time of dosing. In the case of multiple dosing, the cortisol AUC is calculated for a 24-hour period at steady-state conditions. Appendix B gives a detailed description of the equations used.

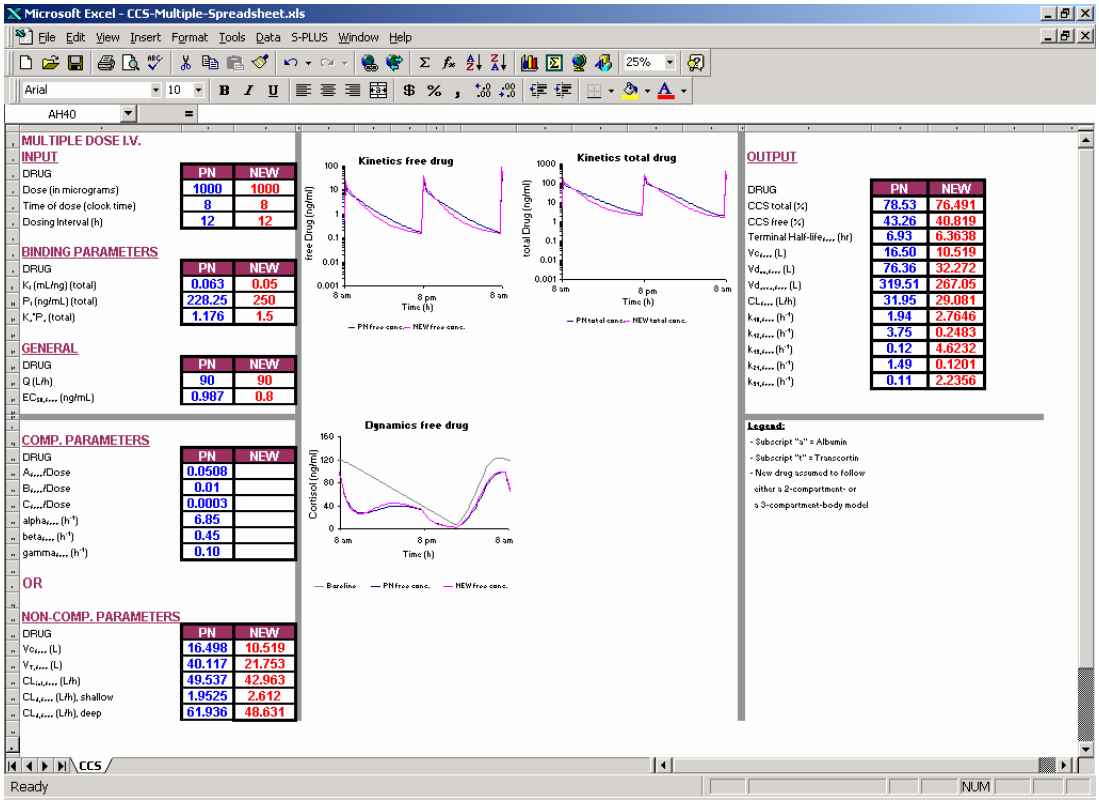

Figure 4-6. Screen capture of the user surface after multiple IV bolus administration

The importance of using the free plasma concentration for a drug showing nonlinear protein binding characteristics is made clear by the following simulation. Using the spreadsheet, total and free plasma concentrations of prednisolone administered in different doses as a single IV bolus are simulated and compared with a constant protein binding value of 94% as it would be seen in linear kinetics. Linear kinetics were chosen for comparison to show the difference in free concentrations if nonlinear binding is not taken into account. The value of 94% is obtained from the literature [159]. Table 4-3 summarizes the plasma concentration for the three situations at t= 0 and Figure 4-7 shows a respective concentration-time profile after administration of 100 mg prednisolone. The model predicted free concentrations could then be linked to simulate the

pharmacodynamic effects of prednisolone. Figure 4-8 shows the result of the simulations for a dose of 100 mg prednisolone.

Table 4-3. Prednisolone plasma concentrations after IV administration of six different doses

Conc. (t=0)/ Dose (µg)	1000	5000	10000	25000	50000	100000
C_t (ng/mL)	295.60	871.03	1538.51	3521.99	6821.03	13416.48
$C_{f,94\%}$ (ng/mL)	17.74	52.26	92.31	211.32	409.26	804.99
C_f (ng/mL)	60.61	303.06	606.12	1515.30	3030.59	6061.18

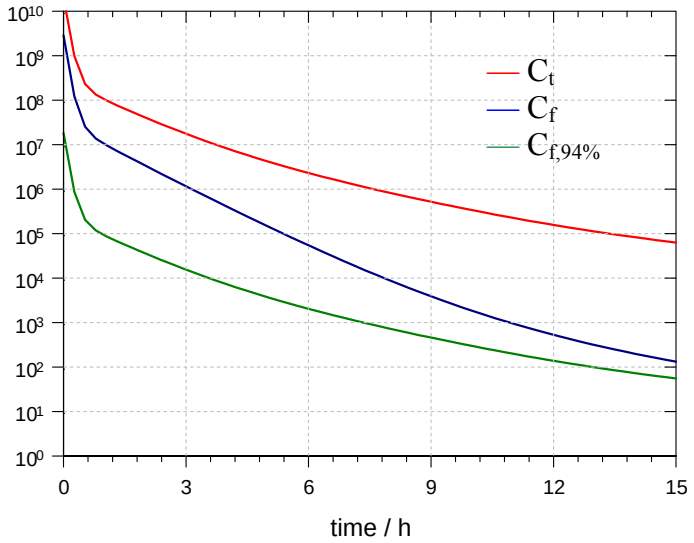

Figure 4-7. Plasma concentration-time profiles after IV administration of 100 mg of prednisolone

Table 4-3 shows a nonlinear relationship between total plasma concentration and administered doses. If the free concentration is calculated using the new approach, a linear relationship between dose and free plasma concentration is seen. This is consistent with general principles of pharmacokinetics.

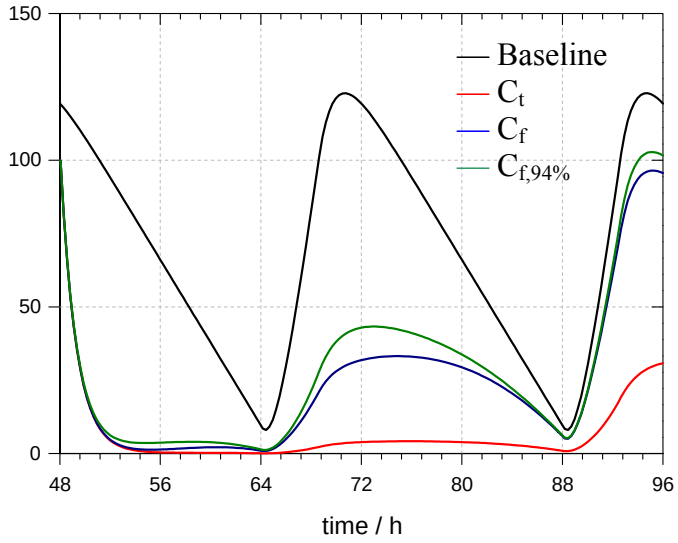

Figure 4-8. Pharmacodynamics after IV administration of 100 mg of prednisolone

In Figure 4-8 the surrogate marker, CCS, is used to quantify the effect of the exogenous corticosteroid. Using the total plasma concentration the value of the CCS is high and therefore, the effect would be overestimated. It would be underestimated using the free plasma concentration under the assumption of linear kinetics. Using the actual free concentration, calculated by the new approach, would result in more accurate concentration-time profiles and hence, a more accurate pharmacodynamic effect.

The difference in the effect between the actual free concentration and the assumed 6% free concentration is not so pronounced because the only parameter changing in Equation 4-11 is the free concentration [43]. However, this approach describes the actual situation better than an assumed linear binding. The accuracy of the predictions is shown in Figure 4-9. It is a plot of model predictions versus actual data after an IV bolus of 49.2 mg prednisolone [44]. The provided spreadsheet allows quick calculation of the expected unbound prednisolone concentrations and, hence, expected systemic effects.

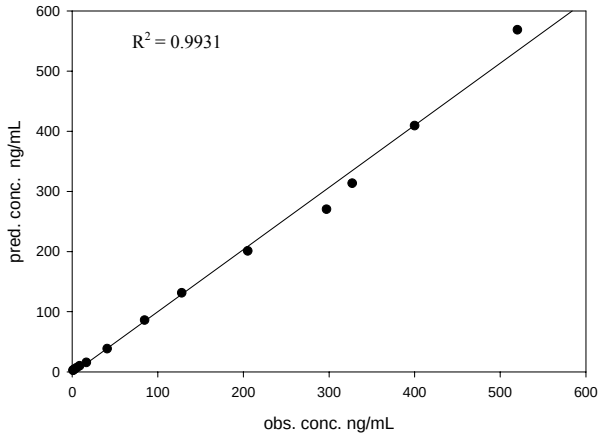

Figure 4-9. Plot of the observed versus predicted plasma-concentrations after IV bolus administration of 49.2 mg prednisolone

If the dose is administered orally rather than intravenously, similar results are obtained. Table 4-4 and Figure 4-10 show the result of the simulations of prednisolone concentrations and concentration-time curves after oral administration of 100 mg prednisolone. Figure 4-11 shows the corresponding pharmacodynamic effect.

Table 4-4. Prednisolone plasma concentrations after oral administration of six different doses

Conc. (t=0)/ Dose (µg)	1000	5000	10000	25000	50000	100000
C_t (ng/mL)	25.41	100.20	163.16	287.38	440.02	707.94
$C_{f,94\%}$ (ng/mL)	1.54	6.01	9.79	17.24	26.40	42.48
C_f (ng/mL)	2.30	11.51	23.01	57.54	115.07	230.14

At therapeutic plasma concentrations, the pharmacokinetics of most drugs can be sufficiently described by first-order linear processes [160]. However, a certain number of drugs show nonlinear pharmacokinetics, making it more difficult to calculate the correct dose and dosing regimen.

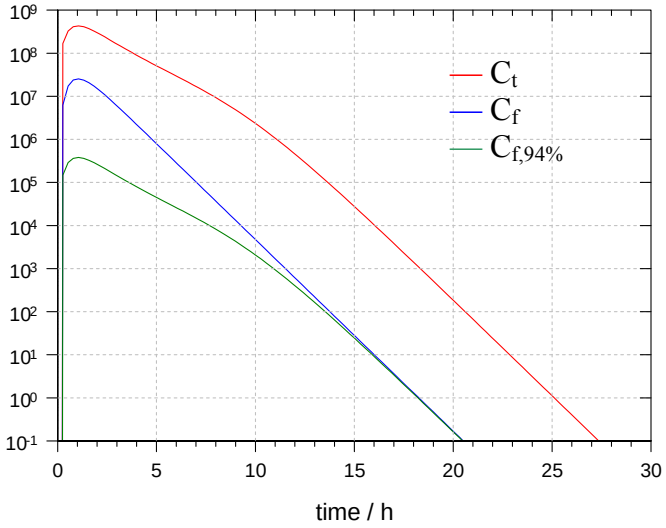

Figure 4-10. Plasma concentration-time profiles after oral administration of 100 mg of prednisolone

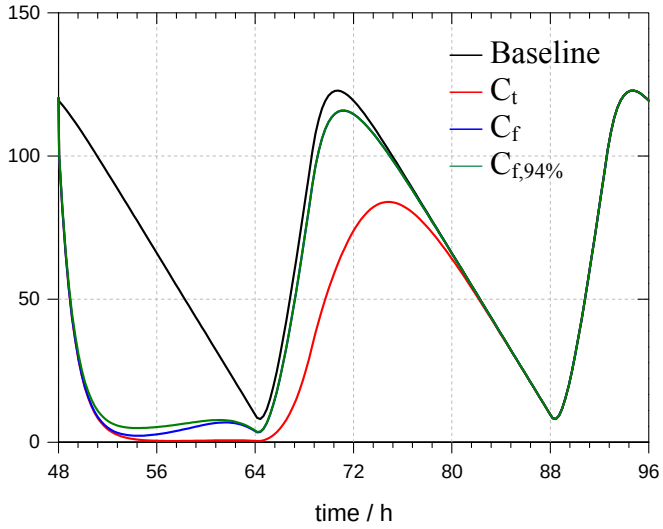

Figure 4-11. Pharmacodynamics after oral administration of 100 mg of prednisolone

With the approach provided, it is possible to simulate the pharmacokinetics, pharmacodynamics, and the corresponding parameters of prednisolone, administered as IV bolus or orally, respectively. It also allows comparisons of prednisolone with any other exogenous corticosteroid showing nonlinear protein binding. Comparisons of an existing or new corticosteroid with prednisolone might be helpful in evaluating the effect as determined by the surrogate marker CCS. According to general principals of

pharmacokinetics, the free concentration is not a constant fraction of the total concentration. Therefore, dosing regimens based on the total concentration or the free fraction are not accurate. Using the free plasma concentration, calculated with the approach provided, results not only in more accurate concentration-time profiles but also more reliable pharmacokinetic parameters because terminal half-life, volume of distribution, and clearance vary in a dose-dependent manner for total drug concentration but are constant for free drug concentrations.

In summary, these four Excel spreadsheets offer a simple way to simulate concentration-time profiles and the systemic effects for a new drug showing nonlinear protein binding. Furthermore, they allow comparison of the results with prednisolone, one of the most commonly prescribed corticosteroids, to assess the effect on the CCS, which, in turn, is related to the side effects profile. It also helps in gaining a better understanding of how nonlinear protein binding affects the pharmacokinetics and pharmacodynamics of prednisolone and other exogenous corticosteroid.

CHAPTER 5
ASSESSMENT OF RECEPTOR BINDING AFFINITY OF LOTEPREDNOL ETABONATE AND OTHER NOVEL SOFT STEROIDS USING A COMPETITIVE BINDING ASSAY IN RATS

Introduction

The main goal of drug design is to find more effective drugs. However, focusing mainly on the increase in effectiveness of a drug might overlook the potential to also increase unwanted side effects. This is particularly true for drugs that act on multiple cell types like corticosteroids [89]. Therefore, drug design should be focused on increasing the therapeutic index, the ratio of efficacy to toxicity. One way to achieve this goal would be the separation of the beneficial and negative effects. Regarding corticosteroids, this is very difficult because all corticosteroid effects (beneficial and side effects) are mediated through the same receptor. It has been tried to increase the binding affinity to the receptors in the tissue where the drug is intended to be effective. This approach has improved therapy over the last years but those drugs still have the ability to produce side effects [59]. Therefore, it is the goal to improve the therapeutic index by also decreasing the negative side effects. Designing predictable metabolic processes into the drug using the soft drug approach seems to improve the therapeutic index better than designing drugs with a high receptor binding affinity and selectivity [89].

For loteprednol etabonate and etiprednol dicloacetate, it could be shown that they have activity and are metabolized into predictable inactive metabolites [67, 161]. For Compounds 929 and D45, it is hypothesized that they show activity at the glucocorticoid receptor because of their structural analogy to loteprednol etabonate. Compounds 902,

453, and 423 are structural analogs of dexamethasone. Therefore, it is hypothesized that they also show high receptor binding affinity.

Due to their predictable metabolism, it is expected that they do not increase the side effect profile and hence, increase the therapeutic ratio.

Materials and Methods

Chemicals

Loteprednol etabonate was kindly provided by Muro Pharmaceuticals Inc. Etiprednol dicloacetate, 929, D45, 902, 453, and 423 were kindly provided by Dr. N. Bodor. ^{3}H-Dexamethasone (Ci/mmol) was purchased from Perkin Life Sciences. Unlabelled dexamethasone was obtained by Fisher Scientific. All other analytical grade chemicals were obtained from Fisher Scientific or equivalent sources. Double distilled deionized water was prepared in our lab (Gainesville, FL).

Animals

The project was approved by the Institutional Animal Care and Use Committee of the University of Florida. The animals, weighing 250 ± 20 g, were obtained from Harlan Sprague Dawley Inc. (IN, USA) and were housed 12 h in a light/dark, constant temperature environment before the experiment.

Dilutions

Various dilutions of loteprednol etabonate, etiprednol dicloacetate, 929, 904, 453, 423, and dexamethasone (0.01 μM, 0.03 μM, 0.1 μM, 0.3 μM, 1 μM, 3 μM, and 10 μM) were prepared using methanol. Due to the low solubility of Compound D45, dilutions were prepared using dimethyl sulfoxide (DMSO). A dexamethasone standard in DMSO was prepared for comparison.

We prepared 100 nM of ^{3}H-labeled dexamethasone (DEX) (10 nM strength in the final incubation mixture) in incubation buffer as tracer solution. A solution of 100 µM unlabeled DEX in ethanol was used to estimate the non-specific binding.

Cytosol Preparation

The rats were anesthetized using a mixture of ketamine (100 mg/mL; Schering-Plough), xylazine (20 mg/mL; BenVenue Lab, Bedford, Ohio) and acepromazine (10 mg/mL; Boehringer Ingelheim, St. Joseph, MO) (3:3:1, v:v) and decapitated. After decapitation, the lungs were removed immediately, weighed, and homogenized with 4 volumes of ice-cold incubation buffer (10 mM Tris/HCL, 10 mM sodium molybdate, 2 mM 1,4-dithioerythritol) in a Virtis 45 homogenizer at 40% of full speed, for three to four periods of 8 s each with a 30 s cooling period in between each step. The resulting homogenate was transferred into centrifuge tubes and incubated with a 5% charcoal suspension (in distilled water) for 10 min to remove endogenous glucocorticoids (volume: 10% of the homogenate). The homogenate was then centrifuged for 20 min at 20,000 g at 4^{o}C in a Beckman centrifuge equipped with a JA-21 rotor (Beckman instruments, Palo Alto, CA) to obtain a clear cytosol. An enzyme inhibitor (diisopropyl fluorophosphate, 5 mM) was added to the cytosol to prevent the cleavage of the ester groups of the compounds by endogenous esterases. Cytosol was always prepared freshly on the day of the experiment.

Cytosol Incubation

A previously published method was used with slight modifications [162]. We added 20 µL of the drug solution or methanol (for control) to prechilled Eppendorf tubes. 20 µL of radioactivity (preparation described above) were then added. Aliquots of the

cytosol (160 µL) were then added. The Eppendorf tubes were vortexed and incubated at 4°C for 16 h.

After the incubation period 200 µL of activated charcoal suspension (5% in distilled water) were added to remove excess radioactivity. The mixture was incubated for 5 min on ice and then centrifuged in a micro-centrifuge (Fisher model 235A) for 5 min at 10,000 rpm. We transferred 300 µL of the supernatant into a scintillation vial and 5 mL of scintillation cocktail added. Then the radioactivity (disintegrations per minute) was determined using a liquid scintillation counter (Beckman model LS 5000 TD, Palo Alto, CA). All experiments were performed in duplicates on three different days.

Data Analysis

The obtained data were analyzed using the software Micro Math Scientist for Windows™ (Version 2.0, Micro Math Inc.) using a standard E_{max}-model with

$$DPM = B_{max} \frac{C^N}{IC_{50}{}^N + C^N} + NS, \qquad (5\text{-}1)$$

where DPM (disintegrations per minute) represents the total tracer binding obtained at any given competitor concentration (C). The data were fitted to the E_{max}-model to obtain the estimates of B_{max} (maximum binding), NS (non-specific binding) and IC_{50} (concentration that produces half of B_{max}). The value of the Hill factor (N) was fixed to 1. Fits were performed individually for a given test compound on a given day. The non-specific binding was estimated using the program and was in close agreement with the non-specific binding obtained in the individual experiments.

In general, dexamethasone was run in all individual experiments (day 1, 2, and 3). The IC_{50} obtained for dexamethasone ($IC_{50,dex}$) was then used to calculate the relative binding affinity (RBA_{test}) of the test compounds from their IC_{50} values ($IC_{50,test}$) as:

$$RBA_{test} = \frac{IC_{50,Dex}}{IC_{50,test}} * 100 . \qquad (5\text{-}2)$$

Results and Discussion

The receptor binding affinities of loteprednol etabonate, etiprednol dicloacetate and other novel soft steroids were determined in freshly prepared rat lung cytosol. All experiments were performed in the presence of the esterase inhibitor diisopropyl fluorophosphate to inhibit degradation to inactive compounds. A representative graphical output of the inhibitory effects of the test compounds on the binding of ^{3}H-dexamethasone is shown in Figure 5-1.

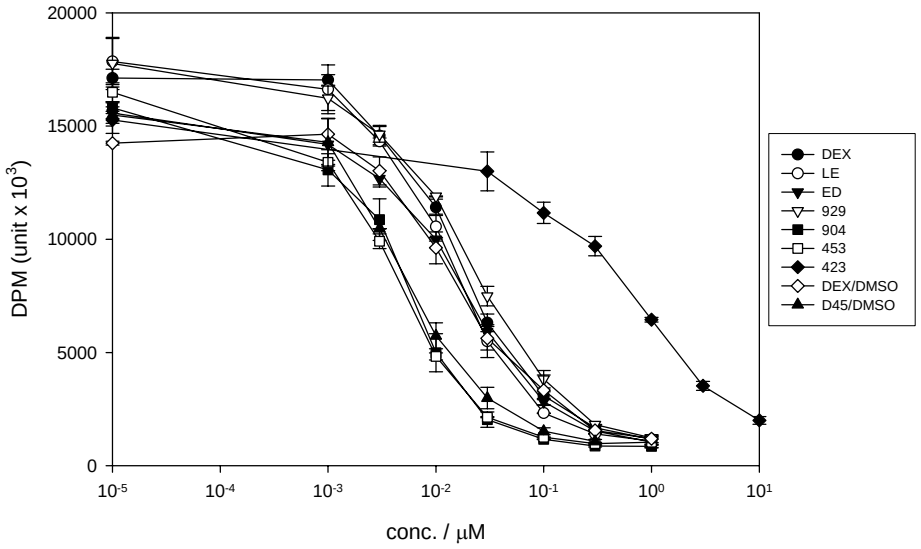

Figure 5-1. Representative graphical output of the competitive binding experiment for loteprednol etabonate, etiprednol dicloacetate and other novel soft steroids

Nonlinear curve fitting was used in all the experiments to determine the IC_{50} values of the different test compounds. The slope factor was fixed to 1, assuming a homogenous

population of binding sites. The resulting receptor binding affinities for all the test compounds are shown in Table 5-1 and Figure 5-2.

Table 5-1. Average relative receptor binding affinities (n=3) of dexamethasone, loteprednol etabonate, etiprednol dicloacetate and other soft steroids

Compound	Relative Receptor Binding Affinity
Dexamethasone*	100
Loteprednol etabonate	129 ± 5.5
Etiprednol dicloacetate	84 ± 16.4
929	85 ± 6.7
904	336 ± 2.5
453	404 ± 16.2
423	3 ± 0
Dexamethasone / DMSO	100
D45 / DMSO	295 ± 47.1

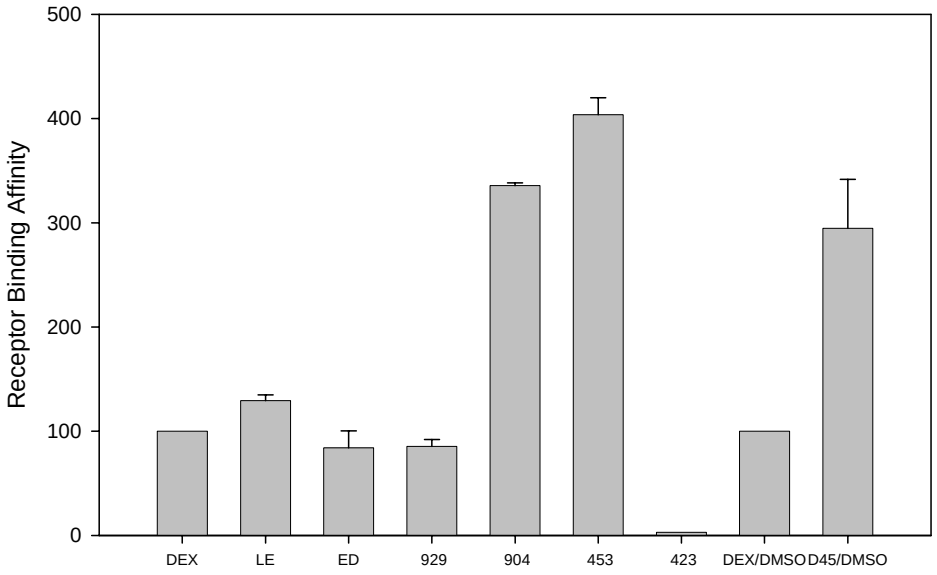

Figure 5-2. Receptor binding affinities of loteprednol etabonate, etiprednol dicloacetate and other novel soft steroids using dexamethasone as reference (RBA_{Dex}=100)

Loteprednol etabonate and its analog compounds 929 and D45 belong to the first generation soft steroids. The synthesis of these drugs is based on the inactive metabolite approach starting from an inactive metabolite of hydrocortisone, cortienic acid [163,

164]. From this lead compound, active compounds were obtained by restoring important pharmacophores found in the 17α and 17β side chains. Two functions were found to be critical for steroid activity. First, a haloester in the 17β-position and second, a carbonate or ether structure in the 17α-position [165]. Loteprednol etabonate and its analogs all have a 17α-ethylcarbonate structure, which resulted in a receptor binding affinity equal to or greater than that of dexamethasone. Introduction of a methyl group in 6α-position (Compound 929) did not lead to the expected increase in affinity to the glucocorticoid receptor. This is most likely because this compound is large and might approach the size-limit of the steroid binding pocket at the receptor. Therefore, it will be increasingly difficult to fit within the binding pocket. However, halogenation (chlorination) of the 9α-position as it has been done for Compound D45 lead to a 2.3-fold increase of the receptor binding affinity compared to loteprednol etabonate. This is in accordance with the general understanding of structure-activity relationships and the effect of halogenation at the 9α-position [166].

Etiprednol dicloacetate and the dexamethasone analogs 904, 453, and 423, belong to the second generation of soft steroids. These compounds have the unique characteristic of halogen substituents at the 17α-position. Buchwald and Bodor [165] found that the halogen containing 17α-pharmacophore overlaps almost perfectly with those of the traditional corticosteroids and that dichlorination seems to be required for activity. One of the explanations for these findings is that due to dichlorination one of the chlorine atoms will lead to pharmacophore overlap while in monochlorinated substituents the chlorine ion points away from the desired direction. In accordance with these findings, Compound 423 shows essentially no binding affinity. The receptor binding affinity of

etiprednol dicloacetate with a 17α-dichlorinated substituent was determined as 84 and was somewhat lower than previously published receptor binding data [165]. However, this difference might be, as mentioned earlier for Compound 929, due to the size of the compound, which makes it more difficult to fit within the binding pocket of the receptor. In general, up to three-fold differences are not uncommon among values determined by different laboratories and these data are mostly within these limits. The Compounds 904 and 453 show a 3.4 to 4-fold higher receptor binding affinity when compared to dexamethasone. The increase in affinity can be explained by halogenation (fluorination) in the 6α and/or 9α-position.

CHAPTER 6
ASSESSMENT OF THE PROTEIN BINDING OF LOTEPREDNOL ETABONATE AND OTHER NOVEL SOFT STEROIDS USING ULTRAFILTRATION

Introduction

Plasma protein binding of drugs has been shown to have significant effects on several aspects of the pharmacokinetics and pharmacodynamics [167]. It is understood, that only the free, unbound drug is pharmacologically active. The nature of the binding can be either specific or unspecific. The binding of cortisol to the α1-glycoprotein transcortin is a case of specific protein binding while binding to albumin is unspecific. In both cases the binding process is reversible and bound and unbound concentrations are in equilibrium.

Protein binding has a great influence on the distribution and elimination behavior of a drug. Binding to plasma proteins, such as albumin, keeps the drug in the blood stream and prevents its diffusion into the tissue. On the other hand, drugs that are mostly unbound in the bloodstream are available for extensive distribution into the tissue. But the extent of distribution is not only dependent on the plasma protein binding but also on the degree of tissue binding. If the tissue binding exceeds the protein binding, the volume of distribution will be large.

The fraction unbound is also important in assessing the clearance of a drug as only the free fraction will be available for elimination. However, corticosteroids are high extraction drugs and therefore, the clearance is only dependent on the liver blood flow.

Ultrafiltration is a commonly used technique to determine the binding of a drug to plasma proteins. The test compound containing plasma is placed on the ultrafiltration device and centrifuged. The filter allows plasma water and small molecules to pass while larger molecules such as proteins are retained. Rapidity, efficiency, and simplicity are some of the advantages of this technique. Therefore, this method has been used to determine the plasma protein binding of loteprednol etabonate, etiprednol dicloacetate, and Compound 929 in diluted human plasma. The plasma protein binding of a drug can be assessed by incubating it with diluted plasma (in this case we used 5% plasma in PBS buffer). The fraction unbound in full plasma can then be calculated from the fraction unbound in diluted plasma. This method is very suitable for drugs that are strongly bound to plasma proteins because almost all of the 5% plasma proteins are bound. It also improves the bioanalytical method because it decreases the interferences of organic compound peaks.

Materials and Methods

Chemicals

Loteprednol etabonate (LE) was provided by Muro Pharmaceutical Inc. Etiprednol dicloacetate (ED) and Compound 929 were kindly provided by Dr. N. Bodor. The internal standard methylprednisolone acetate was purchased from Fisher Scientific. Microcon ultrafiltration devices (Amicon, Millipore corporation, cut off: 30000 molecular weight) were used for the ultrafiltration. Phosphate buffered saline (PBS, pH 7.4) was obtained from Fisher Scientific. Human blank plasma was obtained from the Civitan regional blood system (Gainesville, FL). All other analytical grade chemicals were obtained from Fisher Scientific or equivalent sources. Double distilled deionized water was prepared in our lab (Gainesville, FL).

Preparation of Standard Solutions and Plasma Samples

Primary stock solutions were prepared by dissolving 25 mg of the test compound in 25 mL methanol to obtain a concentration of 1 mg/mL. Further stock solutions of 100 μg/mL, 10 μg/mL, and 1 μg/mL were prepared in a mixture of acetonitrile-water (60:40, v/v). The calibration curve for each compound ranged from 0.1–4 μg/mL. The stock solution for the quality controls was prepared by dissolving 25 mg of test compound in 25 mL methanol. All further stock solutions and quality controls were prepared in mobile phase. The quality controls ranged from 0.1–3 μg/mL. The internal standard (I.S.) methylprednisolone acetate (MPA) was prepared by dissolving 10 mg of MPA in 100 mL methanol to obtain a stock solution with a concentration of 100 μg/mL.

Test samples were prepared by spiking 5% diluted human plasma with different amounts of test compound to obtain total concentrations in plasma ranging from 5 to 20 μg/mL. Quality controls in plasma were prepared by spiking 5% diluted human plasma with different amounts of quality control stock solutions to obtain quality controls in the range of 0.1–3 μg/mL.

Preparation of Diluted Plasma

Human plasma was diluted with phosphate buffered saline (PBS, pH 7.4) to obtain a concentration of 5%.

Ultrafiltration

Spiked human plasma samples were prepared as described earlier. The samples were allowed to equilibrate for 30 min. After equilibration, 0.5 mL of the spiked plasma were transferred onto the Microcon ultrafiltration device (Amicon, Millipore corporation, cut off: 30000 molecular weight) and centrifuged at 4000 g for 1 min (Fisher Scientific Marathon 16 KM). Less than 15% of the total volume was filtered to prevent protein

concentration in the plasma sample. 10 µL of the ultrafiltrate were mixed with 40 µL of mobile phase and 50 µL of internal standard (MPA, 2 µg/mL) and analyzed by HPLC.

The total concentration of drug in plasma was determined from the sample on the top of the filter. For protein precipitation, 100 µL of the sample were mixed with 900 µL acetonitrile and vortexed. The mixture was centrifuged at 7000 rpm for 10 min. 100 µL of the supernatant were mixed with 10 µL of internal standard (MPA, 10 µg/mL) and analyzed by HPLC. All experiments were done in triplicate.

HPCL Conditions

The HPLC consisted of a Shimadzu LC-10AT VP pump, a 50 µL sample loop, a C-8 column (Whatman, 4.6 mm x 25 cm, 5 µm, LOT #2392), an UV-Vis detector at 254 nm (Shimadzu, SPD-10A VP), a Perkin Elmer ISS-100 autosampler, and an Agilent 3396 Series III Integrator. The mobile phase consisted of acetonitrile:water (60:40, v/v) and was pumped at 1.0 mL/min. Calibration curves in mobile phase were linear over the 0.1-4 µg/mL range (r^2>0.98).

Data Analysis

The obtained data were used to determine the amount of drug bound to diluted human plasma proteins using Equation 6-1.

$$C'_{bound} = C'_{total} - C'_{free} \tag{6-1}$$

The fractions bound and unbound can then be calculated using Equation 6-2.

$$f'_{bound} = \frac{C'_{bound}}{C'_{total}} \tag{6-2}$$

$$f_u' = \frac{C_{free}'}{C_{total}'} \quad (6\text{-}3)$$

The free fraction in undiluted plasma (f_u) could then be calculated from the values of the free fraction in 5% diluted plasma (f_u') using Equation 6-4 [113].

$$f_u = \frac{f_u'}{20 - 19 \cdot f_u'} \quad (6\text{-}4)$$

For a complete derivation of this equation see Appendix D [113].

Results and Discussion

The protein binding for all three compounds could be determined in 5% diluted human plasma using the ultrafiltration method. The binding of all three test compounds to plasma proteins was high (>95%) and linear over the entire concentration range tested. The value for loteprednol etabonate was similar to previously determined protein binding values in dog plasma (95.3%) [83].

The results are reported as the fraction unbound in 5% diluted plasma and full human plasma and are summarized in Tables 6-1 through 6-6 and Figures 6-1 through 6-3. The overall mean of the free fraction of loteprednol etabonate, etiprednol dicloacetate, and Compound 929 was 0.031, 0.017, and 0.009, respectively. Therefore, the protein binding is 96.9%, 98.3%, and 99.1%.

Table 6-1. Loteprednol etabonate free fraction in 5% diluted human plasma

Concentration µg/mL	5	7.5	10	15	20
1	0.546	0.350	0.334	0.350	0.350
2	0.435	0.457	0.345	0.391	0.342
3	0.443	0.366	0.341	0.391	0.338
mean	0.481	0.391	0.340	0.377	0.343
SD	0.057	0.058	0.006	0.024	0.006

Table 6-2. Loteprednol etabonate free fraction in full human plasma

Concentration µg/mL	5	7.5	10	15	20
1	0.057	0.026	0.025	0.026	0.026
2	0.037	0.040	0.026	0.031	0.025
3	0.038	0.028	0.025	0.031	0.025
mean	0.044	0.031	0.025	0.029	0.025
SD	0.011	0.008	0.001	0.003	0.001

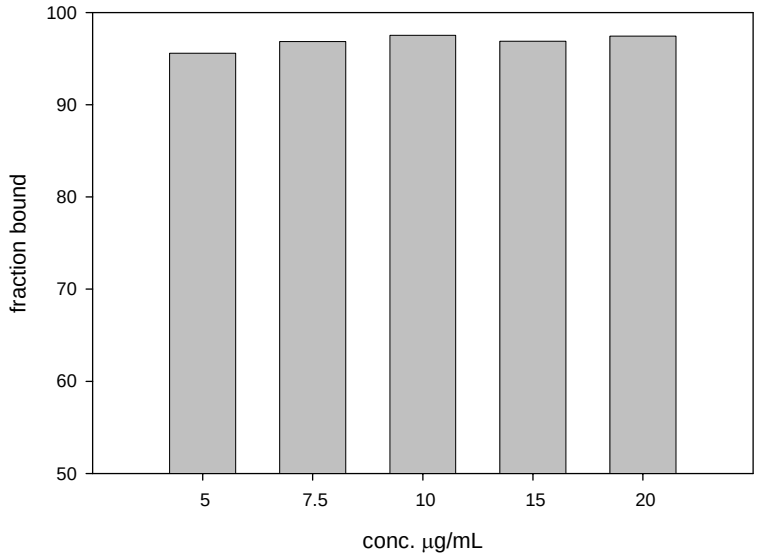

Figure 6-1. Loteprednol etabonate—Fraction bound in full human plasma over a wide concentration range (n=3)

Table 6-3. Etiprednol dicloacetate free fraction in 5% diluted human plasma

Concentration µg/mL	5	7.5	10	15	20
1	0.268	0.245	0.304	0.226	0.247
2	0.249	0.250	0.256	0.214	0.256
3	0.185	0.356	0.254	0.239	0.198
mean	0.234	0.284	0.272	0.226	0.234
SD	0.043	0.063	0.028	0.012	0.031

Table 6-4. Etiprednol dicloacetate free fraction in full human plasma

Concentration µg/mL	5	7.5	10	15	20
1	0.018	0.016	0.021	0.014	0.016
2	0.016	0.016	0.017	0.013	0.017
3	0.011	0.027	0.017	0.015	0.012
mean	0.015	0.020	0.018	0.014	0.015
SD	0.004	0.006	0.003	0.001	0.003

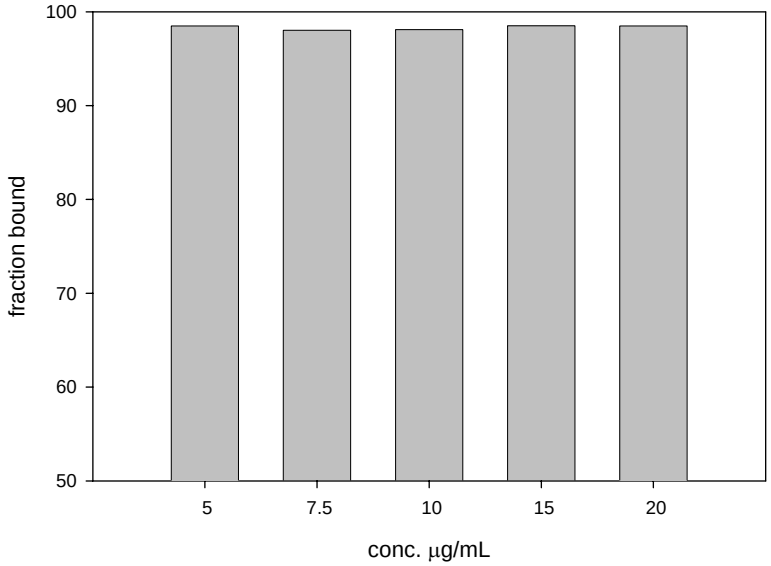

Figure 6-2. Etiprednol dicloacetate—Fraction bound in full human plasma over a wide concentration range (n=3)

Table 6-5. Compound 929 free fraction in 5% diluted human plasma

Concentration µg/mL	5	7.5	10	15	20
1	0.225	0.137	0.126	0.194	0.159
2	0.235	0.109	0.107	0.207	0.127
3	0.225	0.172	0.105	0.159	0.104
mean	0.228	0.140	0.113	0.187	0.130
SD	0.006	0.031	0.012	0.025	0.028

Table 6-6. Compound 929 free fraction in full human plasma

Concentration µg/mL	5	7.5	10	15	20
1	0.014	0.008	0.007	0.012	0.009
2	0.015	0.006	0.006	0.013	0.007
3	0.014	0.010	0.006	0.009	0.006
mean	0.015	0.008	0.006	0.011	0.007
SD	0.000	0.002	0.001	0.002	0.002

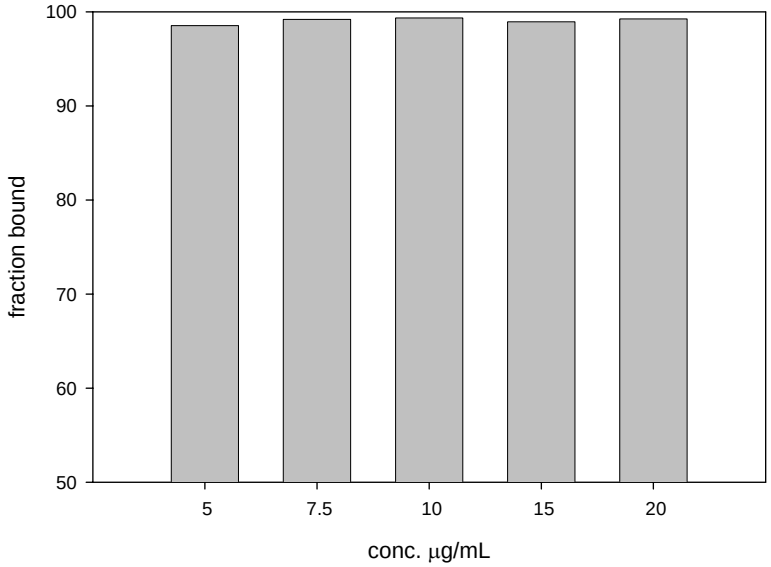

Figure 6-3. Compound 929—Fraction bound in full human plasma of over a wide concentration range (n=3)

Validation

Five-point calibration curves ranging from 0.1-4 µg/mL of each test compound were run each day on three separate days. The calibration curves were linear over the entire concentration range (r^2>0.98). Using linear least square regression, plots were generated, showing the peak-height ratios of the analyte to the internal standard (I.S.) versus the nominal concentration of the calibration standards (Figure 6-4, 6-5, and 6-6).

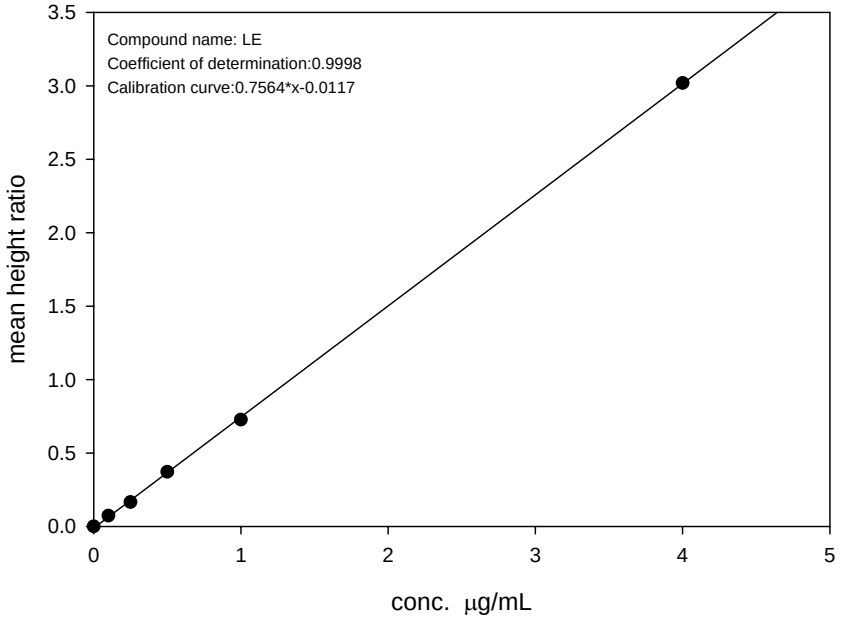

Figure 6-4. LE—representative calibration curve in mobile phase

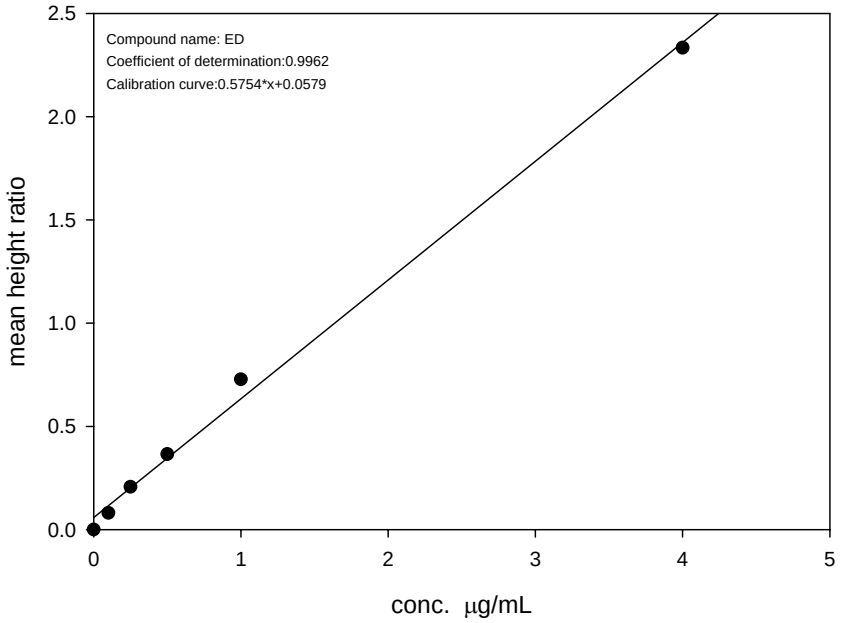

Figure 6-5. ED—representative calibration curve in mobile phase

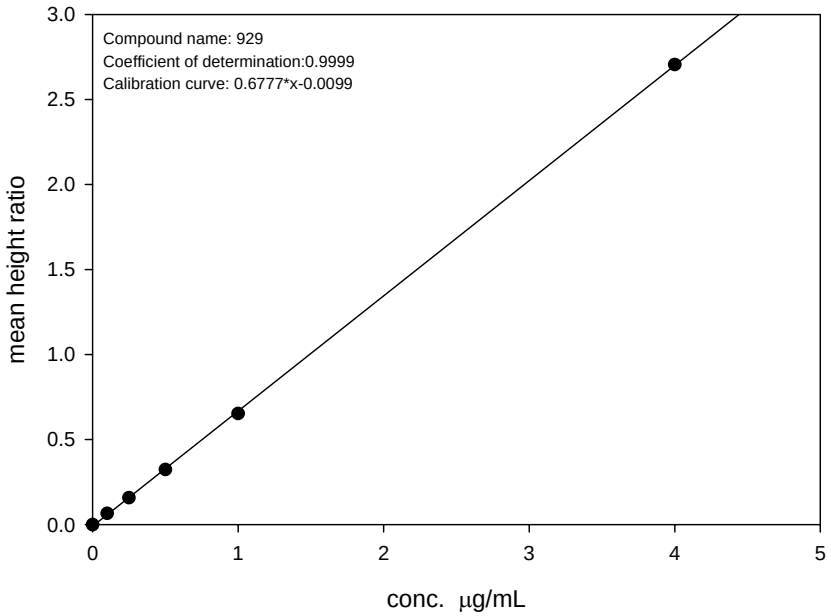

Figure 6-6. Compound 929—representative calibration curve in mobile phase

Four different concentrations, 2.5%, 5%, 10%, and 15%, were used to validate the method of diluted plasma for the determination of plasma protein binding. Each dilution was spiked with test compound to obtain a concentration of 5 μg/mL and 15 μg/mL, respectively. The protein binding was determined for each dilution using ultrafiltration and the results were compared. All experiments were done in triplicate. Figures 6-7 through 6-9 show the fraction bound to proteins for the different plasma dilutions for every test compound. An ANOVA analysis was performed and, on a significance level of 0.05, there was no difference among the four different plasma dilutions for loteprednol etabonate (ANOVA, P=0.33), etiprednol dicloacetate (ANOVA, P=0.33), and Compound 929 (ANOVA, P=0.58).

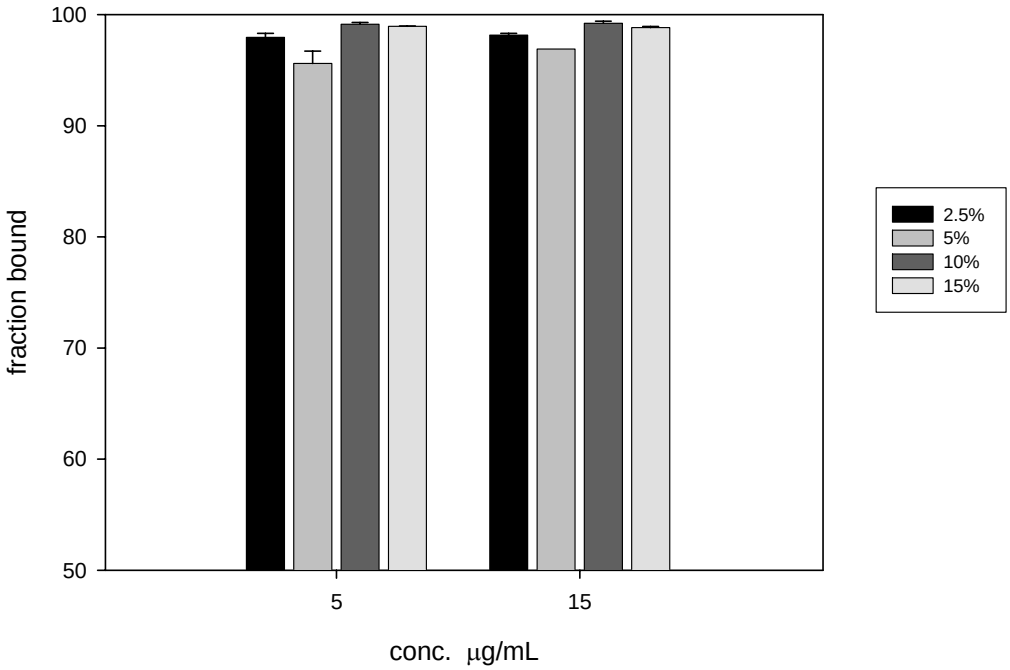

Figure 6-7. Loteprednol etabonate fraction bound in 2.5, 5, 10, and 15% diluted plasma (n=3)

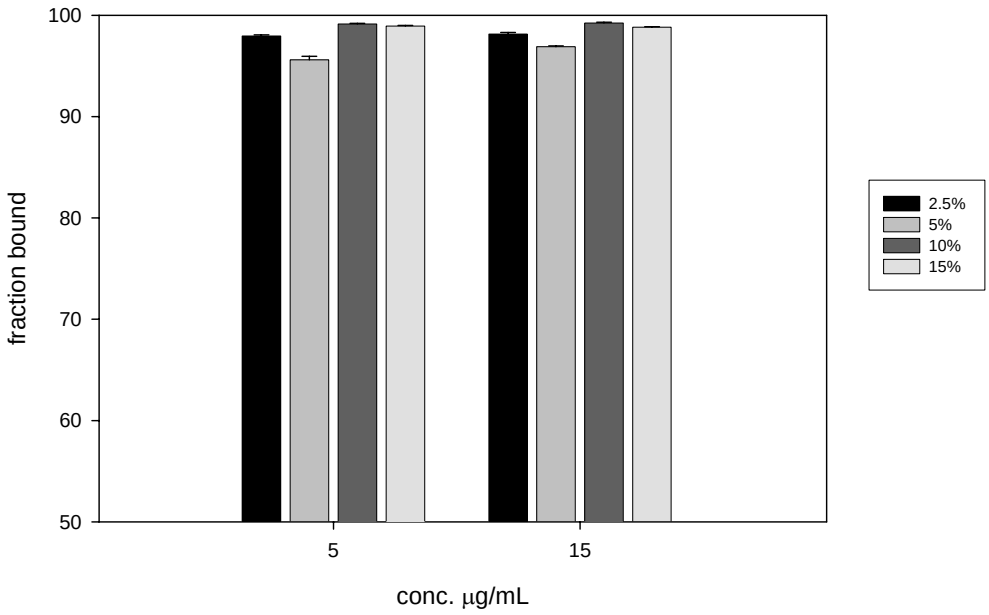

Figure 6-8. Etiprednol dicloacetate fraction bound in 2.5, 5, 10, and 15% diluted plasma (n=3)

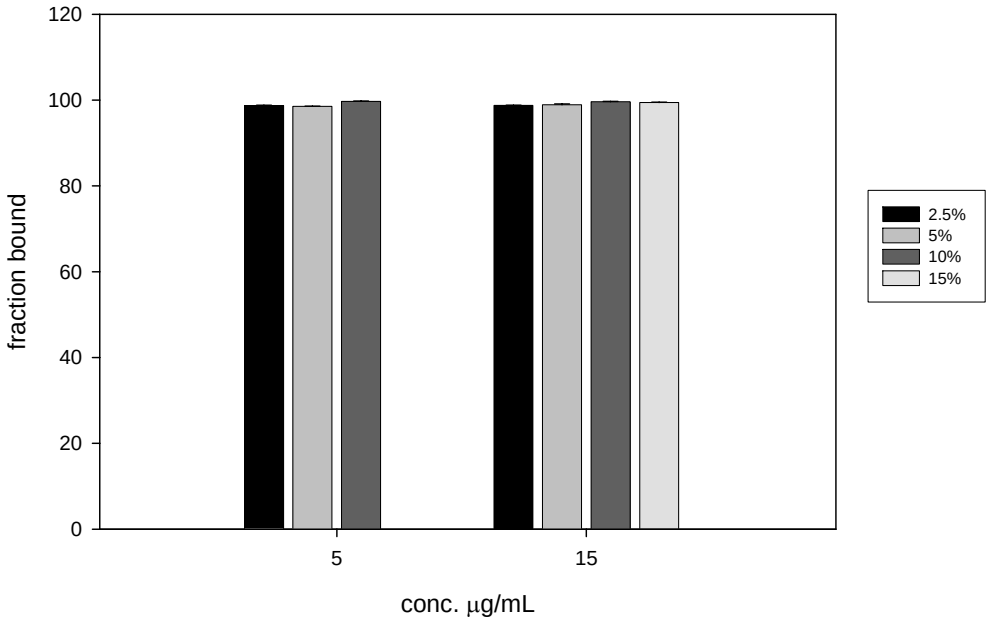

Figure 6-9. Compound 929 fraction bound in 2.5, 5, 10, and 15% diluted plasma (n=3)

All three compounds showed high protein binding and therefore, the free, unbound concentrations in plasma will be low. This might increase the therapeutic ratio by decreasing the chances of these corticosteroids to exert negative systemic side effects.

CHAPTER 7
PHARMACOKINETICS OF A NOVEL SOFT STEROID AFTER IV ADMINISTRATION TO THE RAT

Introduction

Pharmacokinetics describes the fate of the drug once it enters the body. It characterizes its absorption, distribution, metabolism, and excretion. Those characteristics can be described by pharmacokinetic parameters, which can be obtained from the plasma concentrations at various time points after intravenous administration. It is an important part of drug development to assess those parameters as part of the determination of efficacy and toxicity of a new compound.

Compound 929 is a novel soft steroid, which was designed for the treatment of topical inflammations such as asthma bronchiale and allergic rhinitis. Corticosteroids are highly potent anti-inflammatory agents when applied locally. However, the topical administration is often accompanied with undesired systemic side effects. Designing predictable metabolic processes into the drug using the soft drug approach seem to improve the therapeutic ratio. This concept was invented by Bodor [4]. It utilizes structure-metabolic relationships in the design process to control the metabolism of a drug [168]. Applying this concept will lead to a decrease of corticosteroid systemic side effects, and therefore to an increase in the therapeutic ratio (ratio of efficacy to toxicity). One of the major systemic side effects of corticosteroids in humans is the suppression of the hypothalamus-pituitary-adrenal gland axis, which will consequently lead to a

suppression of the endogenous cortisol release. The suppression can be studied by monitoring corticosterone, the major endogenous glucocorticoid in rats.

Materials and Methods

Chemicals

Compound 929 was kindly provided by Dr. N. Bodor. The corticosterone ELISA kit was obtained from Oxford Biomedical Research Inc., Oxford, MI, USA. The rat plasma was purchased from Hilltop lab animals Inc., Scottsdale, PA, USA. All other analytical grade chemicals were obtained from Fisher Scientific or equivalent sources.

Animals

The project was approved by the Institutional Animal Care and Use Committee of the University of Florida. The animals, weighing 350 ± 20 g, were obtained from Harlan Sprague Dawley Inc. (IN, USA) and were housed 12 h in a light/dark, constant temperature environment before the experiment.

Preparation of Standard Solutions

Primary stock solutions were prepared by dissolving 10 mg of Compound 929 in 10 mL methanol to obtain a concentration of 1 mg/mL. Further stock solutions of 100 μg/mL and 10 μg/mL were prepared by diluting the primary stock solution with a mixture of acetonitrile : water (60 :40, v/v). The calibration curve, ranging from 0.25–50 μg/mL, was established by adding known amounts of the stock solutions to pooled rat plasma. Quality controls of 0.25 μg/mL, 6 μg/mL, and 25 μg/mL, were prepared from a separate set of stock solutions. Mometasone fuorate was used as an internal standard. The stock solution of the internal standard was prepared by dissolving 100 μg in 10 mL methanol to obtain a concentration of 10 μg/mL.

Intravenous Administration

Compound 929 was dissolved in a mixture of dimethylsufloxide (DMSO) : polyethylenglycol (PEG) 400 : water (1:8:1, v/v/v) at a concentration of 5 mg/kg and a dosing volume of 1 mL/kg. The non-fasted rats were weighed before the experiment. The weighing was done on a special scale with a cage on it. The rats were anesthetized with isoflurane and the compound was injected into the tail vein using a 26 gauge needle. The procedure had to be done carefully to prevent leakage of the compound solution during the injection.

Blood Collection

Before blood collection rats were anesthetized with isoflurane in an inhalation chamber. For blood collection only a light anesthesia was necessary. The blood collection was not started before the animal was completely anesthetized, which was checked by loss of reflexes. In order to control the depth of the anesthesia, respiratory and pulse rate were checked as well.

Blood was withdrawn by puncturing the vena sublingualis at 0, 5, 15, 30, 60, 90, and 120 min after drug administration. The blood was then collected by a procedure adapted from Zeller et al. [169]. The unconscious rat was held in a supine position and the loose skin at the nape of the neck was gathered up to produce a partial stasis in the venous return from the head. A second person extended the tongue in front with a cotton-tipped applicator stick and carefully pulled it forward with a forceps. The two sublingual veins were clearly visible at the base of the tongue. One of the veins was punctured with a 23 gauge needle. For further blood samples the sublingual veins were punctured alternately and closer to the base of the tongue. After successfully puncturing the sublingual vein, the rat was turned back into a prone position and the blood allowed

to dropping into a tube. As soon as the required volume of blood had been collected, the compression was ceased by releasing the scruff of the neck. The puncture wound was mopped with a cotton-tipped applicator into a 50% solution of iron chloride in order to stop the bleeding.

Sample Extraction

Concentrations of Compound 929 were measured by a modified HPLC method which was established by Bodor et al. [168]. After blood collection, samples were centrifuged at 3000 rpm for 10 min. in a Fisher Scientific Marathon 16 KM centrifuge to obtain the plasma. We mixed 100 µL of plasma with 10 µL of internal standard and vortex mixed. A solution of 200 µL acetonitrile containing 5% dimethylsulfoxide (DMSO) was added and mixed in a vortex mixer. The mixture was then centrifuged at 5000 rpm for 5 min (Fisher Scientific Marathon 16 KM). The supernatant was analyzed by HPLC.

Corticosterone Assay

An ELISA (Enzyme-Linked Immunosorbent Assay) was used for the quantitative analysis of corticosterone levels in rat plasma. This commercially available test kit works on the basis of competition between the enzyme conjugate and the corticosterone in plasma for a limited number of binding sites on an antibody coated plate.

Briefly, 100 µL of plasma were extracted with ethyl ether. The organic phase was transferred into a new vial and the solvent was evaporated to dryness in a vacuum centrifuge. The residue was reconstituted in diluted extraction buffer.

We added 50 µL of sample or standard to the microplate. Diluted enzyme conjugate was added to each well and the mixture was shaken and incubated at room temperature for 1 hour to let competition for the binding sites take place. After incubation, all

remaining unbound material was removed and the bound enzyme conjugate was detected by addition of the substrate, which formed a colorful complex. Quantitative results were obtained by measuring and comparing the absorbance reading of the wells of the samples against the standards at 650 nm. All samples were assayed in duplicate.

HPLC Conditions

The HPLC consisted of a Shimadzu LC-10AT VP pump, a 50 µL sample loop, a C-8 column (Whatman, 4.6 mm x 25 cm, 5 µm, LOT #2392), an UV-Vis detector at 260 nm (Shimadzu, SPD-10A VP), a Perkin Elmer ISS-100 autosampler, and an Agilent 3396 Series III Integrator. The mobile phase consisted of acetonitrile:water (60:40, v/v) and was pumped at 0.8 mL/min. Calibration curves in mobile phase were linear over the 0.25-50 µg/mL range (r^2>0.99).

Data Analysis

Noncompartmental PK analysis was performed using the software WinNonlin® (Version 3.1, Pharsight Corporation, Mountain View, CA, USA). For noncompartmental analysis, the terminal elimination rate constant was determined by linear regression of the natural logarithms of the last n plasma concentrations.

The terminal half-life was determined using Equation 7-1.

$$t_{1/2} = \frac{\ln 2}{k_e} \tag{7-1}$$

The initial concentration at time zero was determined by back-extrapolation. The area under the curve (AUC) was calculated using the trapezoidal rule up to the last data point (C_n) and adding the extrapolated terminal area (Equation 7-2).

$$AUC_{0-\infty} = \sum_{i=1}^{n}\left[\frac{(C_i - C_{i-1})}{2}\cdot(t_i - t_{i-1})\right] + \frac{C_n}{k_e} \tag{7-2}$$

The area under the first moment curve (AUMC) was determined by the trapezoidal rule of the plot $C \cdot t$ versus t up to the last data point (C_n) and adding the extrapolated area, calculated as

$$AUMC_{t\to\infty} = \frac{C_n \cdot t_n}{k_e} + \frac{C_n}{k_e^2}. \quad (7\text{-}3)$$

The mean residence time (MRT), the average time the drug stays in the body, was calculated as the quotient of the AUMC and the AUC.

$$MRT = \frac{AUMC}{AUC} \quad (7\text{-}4)$$

The volumes of distribution of the central compartment (V_c) and at steady-state (Vd_{ss}) were determined as

$$V_c = \frac{Dose}{C_0} \quad (7\text{-}5)$$

$$Vd_{ss} = \frac{Dose \cdot AUMC}{AUC^2}. \quad (7\text{-}6)$$

The total body clearance, CL_{tot}, was determined by the ratio of the dose and the area under the curve.

$$CL = \frac{Dose}{AUC} \quad (7\text{-}7)$$

For compartmental analysis, the software program WinNonlin® (Version 3.1, Pharsight Corporation, Mountain View, CA, USA) was used.

Results and Discussion

Validation

Two eight-point calibration curves ranging from 0.25 to 50 µg/mL of Compound 929 were run each day on three separate days. Using linear least square

regression with and without a weighing scheme ($1/Y^2$), plots were generated, showing the peak-area ratios of the analyte to the internal standard (I.S.) versus the nominal concentration of the calibration standards (Figure 7-1). The residuals improved by weighted ($1/Y^2$) least-squares regression. Best fit of the calibration curve could be achieved by a linear equation y= m*x + b with $1/Y^2$ weighting factor. The correlation coefficient (r^2) was >0.99.

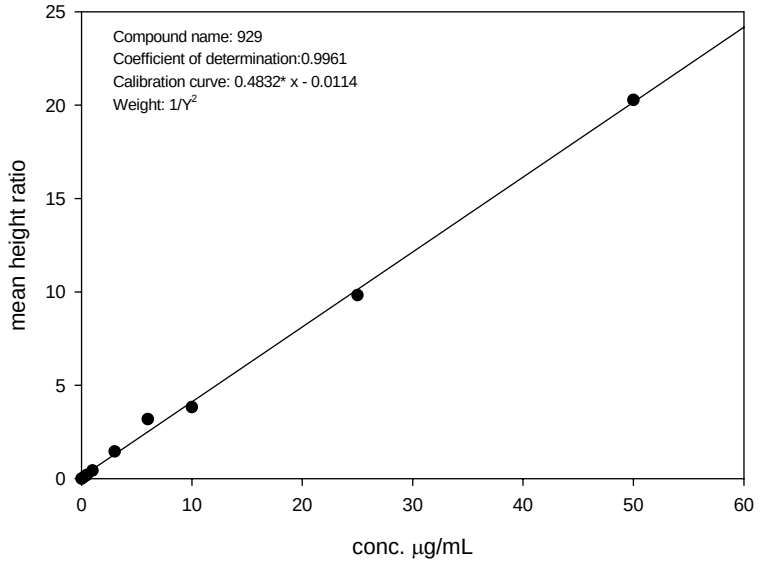

Figure 7-1. Compound 929—Representative calibration curve in plasma

The calibration curves were used to calculate the concentration of independently prepared quality controls. The intra- and inter-batch precision and accuracy data for Compound 929 are shown in Table 7-1 and 7-2.

Table 7-1. Compound 929 standard concentrations—Inter-day precision and accuracy for

Compound 929 µg/mL*								
Theoretical Concentration	0.25	0.5	1	3	6	10	25	50
Mean	0.24	0.51	1.00	3.27	6.03	8.80	25.51	50.97
SD	0.01	0.03	0.01	0.12	0.20	0.36	0.67	0.55
% Bias	-3.00	2.10	-0.03	8.89	0.48	-12.04	2.03	1.93
% CV	3.50	5.09	1.18	3.80	3.39	4.05	1.93	1.08

Table 7-2. Compound 929 quality controls—Inter- and intra-batch precision and accuracy

Theoretical Concentration	0.25	6	25
929 Batch 1 (n=3)			
Mean	0.23	5.61	23.68
SD	0.02	0.4	0.03
% bias	-6.56	-6.49	-5.28
% CV	8.63	7.05	0.13
929 Batch 1 (n=3)			
Mean	0.26	5.81	24.79
SD	0.00	0.01	0.14
% bias	2.46	-3.24	-0.85
% CV	1.2	0.14	0.58
929 Batch 1 (n=3)			
Mean	0.25	6.14	26.77
SD	0.00	0.02	0.04
% bias	-1.42	2.35	7.1
% CV	1.07	0.35	0.17

Pharmacokinetic Analysis

The pharmacokinetics of Compound 929 in rats after intravenous bolus administration of 5 mg/kg was determined. Measurable levels in plasma could be

detected until 60 min after administration. Plasma concentration-time curves suggested that Compound 929 is eliminated in a biphasic manner as shown in Figure 7-2.

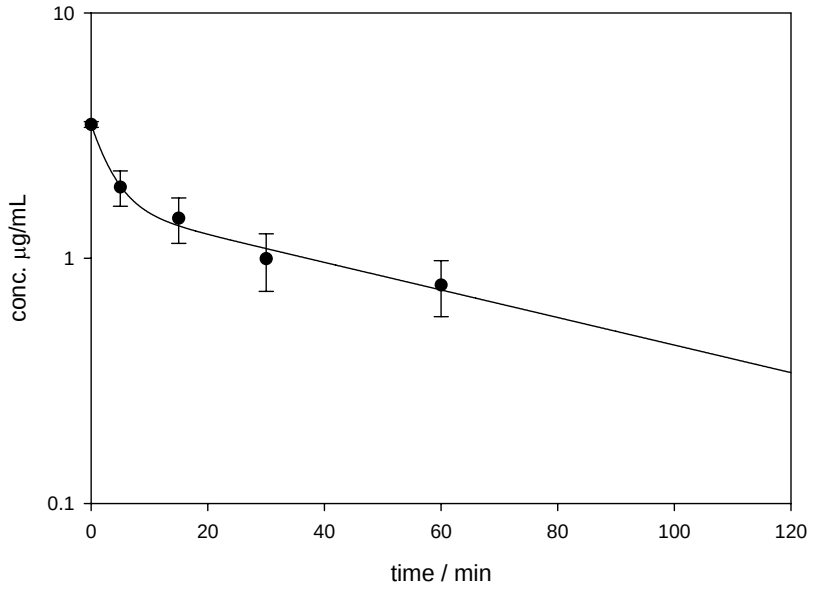

Figure 7-2. Mean plasma concentration-time curve of Compound 929 in rats (n=5) after IV bolus administration of 5 mg/kg

The data shown in Figure 7-2 were analyzed by noncompartmental and compartmental pharmacokinetic analysis. The results of the noncompartmental analysis are shown in Table 7-3. For the compartmental analysis a two-compartment body model described the data very well. The results of the individual pharmacokinetic parameter estimates are shown in Table 7-4.

As can be seen from the tables, the results of the noncompartmental and compartmental analysis are comparable. Compound 929 has a fairly short half-life of 52.3 min as determined by noncompartmental analysis. The clearance value of about 38 mL/min/kg is close to the physiological hepatic blood flow of 58.8 mL/min/kg in the rat, suggesting that Compound 929 is a high extraction drug, mainly cleared by the liver [170]. This is in agreement with currently available corticosteroids, which all have

clearance values of close to or exceeding the hepatic blood flow [171]. A rapid clearance from the systemic circulation will result in low plasma concentrations and therefore decrease the risk for systemic side effects.

The volume of distribution of the central compartment (1430 mL/kg) is similar to the volume of distribution of loteprednol etabonate (1444 mL/kg) after the same IV bolus dose [168]. This is in good agreement with structural analogy of these two compounds.

Table 7-3. Individual pharmacokinetic parameter estimates of Compound 929 after noncompartmental analysis

Parameter/Animal	R1	R2	R6	R8	R11	Mean	SD
C_{max} µg/mL	3.55	3.65	3.40	3.46	3.50	3.51	0.1
CL mL/min/kg	39.1	44.8	50.5	30.4	29.0	38.8	9.2
Vd_{ss} mL/kg	2687	2856	3348	2294	2416	2720	415
V_z mL/kg	2942	3169	3505	2356	2432	2881	488
λ_z min^{-1}	0.013	0.014	0.014	0.013	0.010	0.013	0.001
$t_{1/2}$ min	52.1	49.1	48.1	53.7	58.2	52.3	4.0
AUC min*µg/mL	127.8	111.7	99.0	164.5	172.7	135.1	32.3
AUMC min^2*µg/mL	8779	7124	6565	12413	14418	9860	3421
MRT min	68.7	63.8	66.3	75.5	83.5	71.6	8.0

Table 7-4. Individual pharmacokinetic parameter estimates of Compound 929 after compartmental analysis

Parameter/Animal	R1	R2	R6	R8	R11	Mean	SD
C_{max} µg/mL	3.54	3.60	3.40	3.46	3.50	3.50	0.08
A µg/mL	2.32	2.39	2.17	1.48	1.50	1.97	0.45
B µg/mL	1.22	1.21	1.23	1.98	2.00	1.53	0.42
α min^{-1}	0.142	0.173	0.418	0.447	0.532	0.343	0.174
β min^{-1}	0.009	0.012	0.013	0.012	0.013	0.012	0.002
$t_{1/2}$ (β) min	74.6	58.5	52.6	57.8	53.1	59.3	9.0
k_{10} min^{-1}	0.024	0.031	0.034	0.021	0.022	0.027	0.006
k_{12} min^{-1}	0.073	0.088	0.237	0.178	0.214	0.158	0.074
k_{21} min^{-1}	0.055	0.066	0.160	0.261	0.309	0.170	0.114
CL mL/min/kg	33.8	43.1	50.8	29.7	32.1	37.9	8.8
Vd_c mL/kg	1413	1387	1473	1445	1430	1430	32
Vd_{ss} mL/kg	3263	3230	3657	2430	2417	2999	552
AUC min*µg/mL	148.0	116.0	98.4	168.2	155.7	137.3	29.0
AUMC min^2*µg/mL	14285	8698	7085	13752	11711	11106	3139
MRT min	96.6	75.0	72.0	81.8	75.2	80.1	9.9

Corticosterone Assay

Exogenous corticosteroids are known to suppress the endogenous cortisol levels in humans. The suppression of endogenous cortisol after administration of a synthetic corticosteroid has been used as a surrogate marker to quantify the degree of systemic steroid activity and therefore also of its side effects [155]. The predominant endogenous corticosteroid in the rat is corticosterone. To determine the degree of systemic activity of Compound 929, corticosterone levels were measured before drug administration as well as at each time point of blood collection. Measurement of corticosterone was obtained with a competitive ELISA design. The corticosterone extracted from the plasma sample competed for a limited number of binding sites with the enzyme conjugate. Average corticosterone concentrations for each animal are shown in Table 7-5.

Table 7-5. Average corticosterone concentrations of each animal in ng/mL (n=2) for each time point

time / min	blank	0	5	15	30	60	90	120*
R1	—	159.52 ± 6.46	327.68 ± 44.09	516.86 ± 48.75	648.70 ± 26.26	565.42 ± 41.93	261.74 ± 49.16	533.61 ± 78.92
R2	448.58 ± 54.35	43.15 ± 6.09	460.43 ± 31.05	525.65 ± 0.0	593.38 ± 51.98	338.66 ± 43.30	634.02 ± 47.02	506.59 ± 78.31
R6	482.58 ± 19.53	428.38 ± 20.23	436.99 ± 32.41	579.23 ± 46.85	730.60 ± 4.93	643.33 ± 52.04	632.35 ± 114.57	677.38 ± 100.19
R8	287.95 ± 54.08	168.90 ± 28.85	369.28 ± 0.00	501.15 ± 0.00	442.03 ± 50.59	586.48 ± 78.91	525.86 ± 21.28	487.03 ± 6.57
R11	385.62 ± 92.76	636.33 ± 89.87	683.54 ± 13.83	871.86 ± 123.13	914.92 ± 49.36	1077.04 ± 87.12	1129.11 ± 76.13	862.09 ± 98.68

* 150 min for R1

The physiological serum corticosterone levels in the rat range from 100 ng/mL to 150 ng/mL but can rise over 500 ng/mL upon application of stress [172]. As the table shows, almost all corticosterone concentrations are elevated over the entire time of the experiment. It appears that the control levels as well as the levels after drug administration are elevated, most likely due to isoflurane induced stress at the time of blood sampling.

Validation

Together with the test samples, a standard curve ranging from 0.05 to 5 ng/mL was prepared. Using least square linear regression, a plot was generated showing the percent of maximal binding against the nominal concentration of each standard (Figure 7-3). The calibration curve was linear over the entire range with a correlation coefficient of $r^2 = 0.992$.

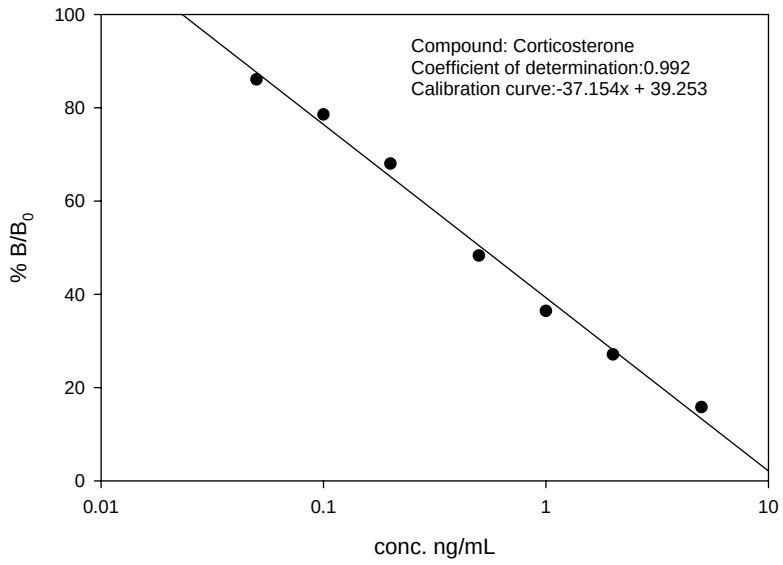

Figure 7-3. Representative calibration curve of an ELISA corticosterone determination

In general, the concept of soft drugs aims to improve the therapeutic ratio of drugs. Soft drugs are intended to be rapidly and predictably metabolized to non-toxic,

non-active moieties after achieving its therapeutic role [4]. Moreover, this inactivation occurs preferably through for example, hydrolysis without the use of enzymes that could be saturated. Therefore, a soft drug would show high concentrations at the site of action but would be inactivated rapidly in the liver once it reaches the systemic circulation. This would lead to an improvement of the therapeutic ratio by minimizing systemic side effects.

The test Compound 929, an analog of the soft steroid loteprednol etabonate, confirms the concept of soft drugs. Plasma concentrations could only be detected until 60 min after drug administration. The pharmacokinetic analysis after IV bolus administration showed a rapid elimination mainly by hepatic metabolism close to the liver blood flow. Additional to its liver metabolism, Compound 929 might also be hydrolyzed by the enzyme pseudo cholinesterase in the blood due to its ester structure at the 17β-position.

Furthermore, Compound 929 seems not to suppress the endogenous corticosterone levels as determined by the ELISA assay. However, these findings would need further confirmation due to the stress induced elevation of corticosterone levels by isoflurane anesthesia at the time of blood sampling.

CHAPTER 8
CONCLUSION

The overall objective of our study was to show that the administration of corticosteroid prodrugs and soft drugs facilitates the use of corticosteroids for both, local and systemic administration. The prodrug and soft drug approach are two different concepts, however, they are both valuable in optimizing corticosteroid therapy. The prodrug methylprednisolone hemisuccinate was administered to patients with acute spinal cord injury to improve their recovery and limit the sensory loss. Due to limited solubility of the active compound methylprednisolone, the prodrug methylprednisolone hemisuccinate was administered to treat SCI. The results have shown that the concentrations of methylprednisolone reach levels in the CSF, the site of action, that are high enough to exert an effect. Therefore, the administration of the prodrug can facilitate the systemic therapy of the corticosteroid methylprednisolone.

Next, the prodrug prednisolone sodium phosphate was used to improve the solubility of the active compound prednisolone to make it suitable for intravenous administration. Prednisolone possesses the special characteristic of nonlinear protein binding. The prodrug and the active metabolite were used to describe the nonlinear pharmacokinetics and pharmacodynamics of this drug and an interactive approach was developed to predict the percent (%) CCS of prednisolone, a good surrogate marker of the systemic activity of corticosteroids. Predicted and measured values correlated very well, validating the approach presented. The spreadsheets are helpful in simplifying and

understanding the effects of nonlinear pharmacokinetics due to saturable protein binding. This might also be valuable in improving dosing and dosing regimens of prednisolone.

Finally, loteprednol etabonate, etiprednol dicloacetate, and other novel soft steroids were investigated for their receptor binding affinity and protein binding, and Compound 929 for its pharmacokinetics after IV bolus administration. The receptor binding affinities of all the test compounds were either similar to or exceeding the binding affinity of dexamethasone. This is in general agreement with their chemical structure and data previously established by Buchwald et. al. [165]. Determination of the receptor binding affinity of corticosteroids is important in assessing the potency due to a positive correlation between the receptor binding affinity and the pharmacological response. However, an increased potency does not necessarily increase the therapeutic ratio, a characteristic of the soft steroids. The lower potency can also be overcome by administration of equivalent doses. Therefore, not only the pharmacodynamics but also the pharmacokinetic properties such as the protein binding must be taken into account.

The protein binding of loteprednol etabonate, etiprednol dicloacetate, and Compound 929 to human plasma proteins was assessed. The free fractions of the test compounds were less than 0.04, indicating a high binding to human plasma proteins. The results for loteprednol etabonate are similar to a study in dog plasma, which resulted in a plasma protein binding of around 95% [83]. The small percentage of free, unbound drug of all three test compounds will decrease their risk to exert unwanted systemic side effects since it is understood that only this free fraction is pharmacologically active.

The pharmacokinetics after IV bolus administration of Compound 929 were assessed to characterize its distribution, metabolism and excretion. It could be shown that

the compound is metabolized and excreted rapidly from the blood stream with a total clearance volume close to the liver blood flow. In addition to its hepatic inactivation, Compound 929 might be already metabolized by pseudo cholinesterase in the blood stream due to its ester structure. The rapid inactivation is an advantage of the soft steroids over currently available corticosteroids because systemic side effects can be minimized and the therapeutic ratio can be increased.

In summary, it could be shown that the prodrugs methylprednisolone hemisuccinate and prednisolone sodium phosphate facilitate the systemic administration of corticosteroids. Both drugs are important anti-inflammatory and immunosuppressive agents in the treatment of acute spinal cord injury and several diseases, including rheumatoid arthritis and shock symptoms, respectively.

The investigated soft steroids showed moderate to high receptor binding affinity, high protein binding to human plasma proteins, and a rapid inactivation after IV bolus administration, characteristics that improve the therapeutic ratio. This will be helpful for the development of safer corticosteroids in the treatment of asthma and allergic rhinitis.

APPENDIX A
NONMEM CODE FOR PK MODEL

```
$PROBLEM PRODRUG AND DRUG IN PLASMA AND CSF
$INPUT ID TIME AMT DV RATE CMT MDV
$DATA MPHSMPPC7.CSV IGNORE=C
$SUBROUTINE ADVAN9 TRANS 1 TOL=3

$MODEL
COMP=(CENTRAL1)
COMP=(PERIPH1)
COMP=(CENTRAL2)
COMP=(PERIPH2)

$PK
TVKF    = THETA(1)
TVV1    = THETA(2)
TVK12   = THETA(3)
TVK21   = THETA(4)
TVK2    = THETA(5)
TVV2    = THETA(6)
TVK34   = THETA(7)
TVK43   = THETA(8)

KF=TVKF*EXP(ETA(1))
V1=TVV1*EXP(ETA(2))
K12=TVK12*EXP(ETA(3))
K21=TVK21*EXP(ETA(4))
K2=TVK2*EXP(ETA(5))
V2=TVV2*EXP(ETA(6))
K34=TVK34*EXP(ETA(7))
K43=TVK43*EXP(ETA8))

IF      (KF.LT.0.0001) EXIT 1 15
IF      (KF.GT.30)  EXIT 1 15
IF      (V1.LT.0.001) EXIT 1 15
IF      (V1.GT.500)  EXIT 1 15
IF      (K12.LT.0.00001) EXIT 1 25
IF      (K12.GT.10)  EXIT 1 25
IF      (K21.LT.0.00001) EXIT 1 35
IF      (K21.GT.10)  EXIT 1 35
IF      (K2.LT.0.0001) EXIT 1 45
```

```
IF      (K2.GT.30)  EXIT 1 45
IF      (V3.LT.0.001) EXIT 1 55
IF      (V3.GT.500)  EXIT 1 55
IF      (K34.LT.0.001) EXIT 1 65
IF      (K34.GT.10)  EXIT 1 65
IF      (K43.LT.0.001) EXIT 1 75
IF      (K43.GT.10)  EXIT 1 75

S1=V1
S3=V3
S4=1.76

$DES
DADT(1)=-KF*A(1)+K21*A(2)-K12*A(1)
DADT(2)=-K21*A(2)+K12*A(1)
DADT(3)=KF*A(1)-(K2+K34)*A(3)+K43*A(4)
DADT(4)=K34*A(3)-K43*A(4)

$THETA
(0.0001, 0.412); KF
(0.001, 47.3); V1
(0.00001, 0.311); K12
(0.00001, 0.363); K21
(0.5, 1.07, 2.0); K2
(20.0, 30.1, 40.0); V3
(0.001, 0.030); K34
(0.001, 0.246); K43

$OMEGA
(0.308); FOR KF
(0.308); FOR V1
(0.308); FOR K12
(0.308); FOR K21
(0.308); FOR K2
(0.308); FOR V3
(0.35); FOR K34
(0.35); FOR K43

$ERROR
R1=0
IF (CMT.EQ.1)  R1=1
R2=0
IF (CMT.EQ.3)  R2=1
R3=0
IF (CMT.EQ.4)  R3=1
```

```
Y1=F*EXP(EPS(1))
Y2=F*EXP(EPS(2))
Y3=F*EXP(EPS(3))

Y=R1*Y1+R2*Y2+R3*Y3

IPRED=F
IRES=DV-IPRED

$SIGMA
(2.364);        MPHS-PLASMA
(0.3725);       MP-PLASMA
(0.3748);       MP-CSF

$ESTIMATION METHOD=1 MAXEVAL=9999 PRINT=5 NOABORT; MSFO=msf1
$TABLE ID TIME AMT KF V1 K12 K21 K2 V3 K34 K43 CMT IPRED
NOPRINT ONEHEADER FILE=MPHSnew9.TAB
```

APPENDIX B
ALGORITHM FOR CUMULATIVE CORTISOL SUPPRESSION

This appendix gives an overview of the algorithm used to calculate the cumulative cortisol suppression of prednisolone and/or any other steroid after IV and oral administration using Microsoft Excel®.

- Scale

Parameter used to divide the time intervals equally over 1000 time points. It is calculated by giving the first cell the value zero. The value of the next cell (same column) is calculated by adding 1 to the previous cell and so on until 1000 time points are generated.

- Absolute Time

Generates 1000 time points.

T_{abs}= Scale/1000 * Number of hours of simulation

Let M be the number of 24-hour intervals in the simulation.

If $T_{abs} \leq T_{max}$ (time of maximum release of cortisol), then M = 0; otherwise

Truncate$\{(T_{abs} - T_{max})/24+1]$, where "Truncate" truncates a number to an integer by removing the fractional part of the number.

- Time

$$t = T_{abs} - (M \cdot 24) \qquad \text{(B-1)}$$

- Cortisol release

As described earlier, the daily cortisol release at baseline can be expressed with two straight-line equations (Equation 4-8 and 4-9).

IF $T_{min} \geq t$, THEN let F1 = 1; otherwise F1 = 0.

Similarly, IF $T_{min} \leq t$, THEN let F2 = 1; otherwise F2 = 0.

- Release rate before drug administration

$$RR = F1 \cdot Rc_1 + F2 \cdot Rc_2 \quad \text{(B-2)}$$

- Release rate after drug administration

$$RR_{drug} = RR \cdot \Delta t \cdot \left[1 - E_{\max} \cdot C / (EC_{50} + C\right] \quad \text{(B-3)}$$

where Δt = time interval between two adjacent times.

- Amount of cortisol eliminated over time, calculated using the trapezoidal rule

$$E_t = CL^{Cort} \cdot \left[C_j + C_{j+1}\right] / 2 \cdot \Delta t \; ; \; (C_0 = 120 ng / mL) \quad \text{(B-4)}$$

where C_j and C_{j+1} are concentrations at times j and j+1.

- Plasma cortisol concentrations before drug administration

$$C_{baseline} = \left\lfloor C_j \cdot V_d + \left(RR \cdot \Delta t\right) - E_t \right\rfloor / V_d \; ; \text{(ng/mL)} \quad \text{(B-5)}$$

- Plasma cortisol concentration after drug administration

$$C_{drug} = \left\lfloor C_j \cdot V_d + \left(RR_{drug} \cdot \Delta t\right) - E_t \right\rfloor / V_d \; ; \text{(ng/mL)} \quad \text{(B-6)}$$

- Calculation of number of doses N

Until the time of administration of the dose, the concentration of the drug C= 0.

IF $\{T_{adm}$ + (Dosing interval (τ) * Total number of doses)$\} \leq T$

THEN N = INTEGER $\{(T-T_{adm}) / \tau+1\}$

- Time (T_{time}) to be used for calculating drug concentration

IF $T < T_{adm}$ then $T_{time} = 0$, ELSE $T_{time} = \{T - T_{adm} - (N-1) * \tau\}$

Determination of the PK output parameter half-life, volumes of distribution, clearance, and rate constants for prednisolone after IV administration.

- Terminal half-life (h)

$$t_{1/2,\,free} = \frac{0.693}{\gamma_{free}} \tag{B-7}$$

- Volumes of distribution (L)

$$V_{C,\,free} = \frac{D}{(A_{free} + B_{free} + C_{free})} \tag{B-8}$$

$$Vd_{ss,\,free} = \left(1 + \frac{k_{12,\,free}}{k_{21,\,free}} + \frac{k_{13,\,free}}{k_{31,\,free}}\right) \cdot V_{C,\,free} \tag{B-9}$$

$$Vd_{area,\,free} = \frac{CL_{free}}{\gamma_{free}} \tag{B-10}$$

- Clearance (L/h)

$$CL_{free} = k_{10,\,free} \cdot V_{C,\,free} \tag{B-11}$$

- Rate constants (1/h)

$$k_{10,\,free} = \frac{\alpha_{free} \cdot \beta_{free} \cdot \gamma_{free}}{k_{21,\,free} \cdot k_{31,\,free}} \tag{B-12}$$

$$k_{12,\,free} = \alpha_{free} + \beta_{free} + \gamma_{free} - \left(k_{10,\,free} + k_{13,\,free} + k_{31,\,free} + k_{21,\,free}\right) \tag{B-13}$$

$$k_{13,\,free} = \frac{\left(\beta_{free} \cdot \gamma_{free} + \alpha_{free} \cdot \beta_{free} + \alpha_{free} \cdot \gamma_{free}\right) - k_{31,\,free}\left(\alpha_{free} + \beta_{free} + \gamma_{free}\right) -}{k_{21,\,free} - k_{31,\,free}}$$

$$\frac{k_{10,\,free} k_{21,\,free} + k_{31,\,free}^{\,2}}{k_{21,\,free} - k_{31,\,free}} \tag{B-14}$$

$$k_{21,\,free} = \text{ from curve fitting} \tag{B-15}$$

$$k_{31,\,free} = \text{ from curve fitting} \tag{B-16}$$

Determination of the PK output parameter for the new drug after IV administration. The parameter can be calculated in two different ways, depending on the input parameters (compartmental or noncompartmental).

PK output parameter on the basis of a two-compartment body model:

- Terminal half-life (h)

$$t_{1/2,\,free} = \frac{0.693}{\beta_{free}} \tag{B-17}$$

- Volumes of distributions (L)

$$V_{C,\,free} = \frac{D}{C_{0,\,free}} \tag{B-18}$$

$$Vd_{ss,\,free} = \left(1 + \frac{k_{12,\,free}}{k_{21,\,free}}\right) \cdot V_{C,\,free} \tag{B-19}$$

$$Vd_{area,\,free} = \frac{CL_{free}}{\beta_{free}} \tag{B-20}$$

- Clearance (L/h)

$$CL_{free} = k_{10,\,free} \cdot V_{C,\,free} \tag{B-21}$$

- Rate constants (1/h)

$$k_{10,\,free} = \frac{\alpha_{free} \cdot \beta_{free}}{k_{21,\,free}} \tag{B-22}$$

$$k_{12,\,free} = \left(\alpha_{free} + \beta_{free} - k_{10,\,free} - k_{21,\,free}\right) \tag{B-23}$$

$$k_{21,\,free} = \frac{A_{free} \cdot \beta_{free} + B_{free} \cdot \alpha_{free}}{A_{free} + B_{free}} \tag{B-24}$$

PK output parameter on the basis of three-compartment body model:

- Terminal half-life (h)

$$t_{1/2,\,free} = \frac{0.693}{\gamma_{free}} \tag{B-25}$$

- Volumes of distributions (L)

$$V_{C,\,free} = \frac{D}{C_{0,\,free}} \tag{B-26}$$

$$Vd_{ss,\,free} = \left(1 + \frac{k_{12,\,free}}{k_{21,\,free}} + \frac{k_{13,\,free}}{k_{31,\,free}}\right) \cdot V_{C,\,free} \tag{B-27}$$

$$Vd_{area,\,free} = \frac{CL_{free}}{\gamma_{free}} \tag{B-28}$$

- Clearance (L/h)

$$CL_{free} = k_{10,\,free} \cdot V_{C,\,free} \tag{B-29}$$

- Rate constants (1/h)

$$k_{10,\,free} = \frac{\alpha_{free} \cdot \beta_{free} \cdot \gamma_{free}}{k_{21,\,free} \cdot k_{31,\,free}} \tag{B-30}$$

$$k_{12,\,free} = \frac{\left(\beta_{free} \cdot \gamma_{free} + \alpha_{free} \cdot \beta_{free} + \alpha_{free} \cdot \gamma_{free}\right) - k_{21,\,free}\left(\alpha_{free} + \beta_{free} + \gamma_{free}\right) - k_{10,\,free} \cdot k_{31,\,free} + {k_{21,\,free}}^2}{k_{31,\,free} - k_{21,\,free}} \tag{B-31}$$

$$k_{21,\,free} = 0.5 \cdot \left[-\left[-\frac{\alpha_{free}C_{free} + \alpha_{free}B_{free} + \gamma_{free}A_{free} + \gamma_{free}B_{free} + \beta_{free}A_{free} + \beta_{free}C_{free}}{A_{free} + B_{free} + C_{free}} + \sqrt{\left(\frac{\alpha_{free}C_{free} + \alpha_{free}B_{free} + \gamma_{free}A_{free} + \gamma_{free}B_{free} + \beta_{free}A_{free} + \beta_{free}C_{free}}{A_{free} + B_{free} + C_{free}}\right)^2 - 4 \cdot \left(\frac{\alpha_{free}\beta_{free}C_{free} + \alpha_{free}\gamma_{free}B_{free} + \beta_{free}\gamma_{free}A_{free}}{A_{free} + B_{free} + C_{free}}\right)}\right]\right] \tag{B-32}$$

$$k_{13,\,free} = \left(A_{free} + B_{free} + C_{free}\right) - \left(k_{10,\,free} + k_{12,\,free} + k_{21,\,free} + k_{31}\right) \tag{B-33}$$

$$k_{31,free} = 0.5 \cdot \left[-\left[-\frac{\alpha_{free} C_{free} + \alpha_{free} B_{free} + \gamma_{free} A_{free} + \gamma_{free} B_{free} + \beta_{free} A_{free} + \beta_{free} C_{free}}{A_{free} + B_{free} + C_{free}} - \sqrt{\left(\frac{\alpha_{free} C_{free} + \alpha_{free} B_{free} + \gamma_{free} A_{free} + \gamma_{free} B_{free} + \beta_{free} A_{free} + \beta_{free} C_{free}}{A_{free} + B_{free} + C_{free}} \right)^2 - 4 \cdot \left(\frac{\alpha_{free} \beta_{free} C_{free} + \alpha_{free} \gamma_{free} B_{free} + \beta_{free} \gamma_{free} A_{free}}{A_{free} + B_{free} + C_{free}} \right)} \right] \right] \quad \text{(B-34)}$$

PK output parameter on the basis of noncompartmental analysis:

- Terminal half-life (h)

$$t_{1/2} = \frac{0.693}{\beta_{free}} \qquad \text{for a two-compartment body model} \quad \text{(B-35)}$$

or

$$t_{1/2} = \frac{0.693}{\gamma_{free}} \qquad \text{for a three-compartment body model} \quad \text{(B-36)}$$

- Volumes of distribution (L)

$$V_{C,free} = parameter$$

$$Vd_{ss.free} = \left(1 + \frac{k_{12,free}}{k_{21,free}} + \frac{k_{13,free}}{k_{31,free}} \right) \cdot V_{C,free} \quad \text{(B-37)}$$

$$Vd_{area,free} = \frac{CL_{free}}{\beta_{free}} \qquad \text{for a two-compartment body model} \quad \text{(B-38)}$$

or

$$Vd_{area,free} = \frac{CL_{free}}{\gamma_{free}} \qquad \text{for a three-compartment body model} \quad \text{(B-39)}$$

- Clearance (L/h)

$$CL_{free} = \frac{CL_{\text{int},free} \cdot Q}{CL_{\text{int},free} + Q} \quad \text{(B-40)}$$

- Rate constants (1/h)

$$k_{10,\,free} = \frac{CL_{free}}{V_{C,\,free}} \tag{B-41}$$

$$k_{12,\,free} = \frac{CL_{d,\,free,s}}{V_{C,\,free}} \tag{B-42}$$

$$k_{21,\,free} = \frac{CL_{d,\,free,s}}{V_{T,\,free}} \tag{B-43}$$

$$k_{13,\,free} = \frac{CL_{d,\,free,d}}{V_{C,\,free}} \tag{B-44}$$

$$k_{31,\,free} = \frac{CL_{d,\,free,d}}{V_{T,\,free}} \tag{B-45}$$

- Macro-constants (ng/mL)

$$A_{free} = \frac{D \cdot \left(\alpha_{free} - k_{21,\,free}\right)}{V_{C,\,free} \cdot \left(\alpha_{free} - \beta_{free}\right)} \quad \text{for a two-compartment body model} \tag{B-46}$$

or

$$A_{free} = \frac{D \cdot \left(k_{21,\,free} - \alpha_{free}\right) \cdot \left(k_{31,\,free} - \alpha_{free}\right)}{V_{C,\,free} \cdot \left(\alpha_{free} - \beta_{free}\right) \cdot \left(\alpha_{free} - \gamma_{free}\right)} \quad \text{for a three-compartment body model} \tag{B-47}$$

$$B_{free} = \frac{D \cdot \left(k_{21,\,free} - \beta_{free}\right)}{V_{C,\,free} \cdot \left(\alpha_{free} - \beta_{free}\right)} \quad \text{for a two-compartment body model} \tag{B-48}$$

or

$$B_{free} = \frac{D \cdot \left(k_{21,\,free} - \beta_{free}\right) \cdot \left(\beta_{free} - k_{31,\,free}\right)}{V_{C,\,free} \cdot \left(\alpha_{free} - \beta_{free}\right) \cdot \left(\beta_{free} - \gamma_{free}\right)} \quad \text{for a three-compartment body model} \tag{B-49}$$

$$C_{free} = \frac{D \cdot \left(k_{21,\,free} - \gamma_{free}\right) \cdot \left(k_{31,\,free} - \gamma_{free}\right)}{V_{C,\,free} \cdot \left(\alpha_{free} - \gamma_{free}\right) \cdot \left(\beta_{free} - \gamma_{free}\right)} \tag{B-50}$$

$$\alpha_{free} = \frac{k_{10,\,free} \cdot k_{21,\,free}}{\beta_{free}} \quad \text{for a two-compartment body model} \tag{B-51}$$

or

α_{free} = solution of the cubic equation for a three-compartment body model (B-52)

$$\beta_{free} = 0.5 \cdot \left[\left(k_{10,free} + k_{12,free} + k_{21,free} \right) - \sqrt{\left(k_{10,free} + k_{12,free} + k_{21,free} \right)^2 \cdot 4 \cdot k_{21,free} \cdot k_{10,free}} \right]$$

for a two-compartment body model (B-53)

β_{free} = solution of the cubic equation for a three-compartment body model (B-54)

γ_{free} = solution of the cubic equation (B-55)

- Cubic equation to solve for $\alpha_{free}, \beta_{free}, \gamma_{free}$:

$$S^3 + pS^2 + qS + r = 0 \quad \text{(B-56)}$$

$$p = k_{10,free} + k_{12,free} + k_{13,free} + k_{21,free} + k_{31,free} \quad \text{(B-57)}$$

$$q = (k_{10,free} + k_{12,free} + k_{13,free}) \cdot k_{21,free} + \left(k_{10,free} + k_{12,free} + k_{13,free} \right) \cdot k_{31,free} + k_{21,free} \cdot k_{31,free} - k_{12,free} \cdot k_{21,free} - k_{13,free} \cdot k_{31,free} \quad \text{(B-58)}$$

$$r = (k_{10,free} + k_{12,free} + k_{13,free}) \cdot k_{21,free} \cdot k_{31,free} - k_{12,free} \cdot k_{21,free} \cdot k_{31,free} - k_{13,free} \cdot k_{31,free} \cdot k_{21,free} \quad \text{(B-59)}$$

$$\Theta = a\cos\left(\frac{-\frac{b}{2}}{\left(\frac{-a^3}{27} \right)^{0.5}} \right) \quad \text{(B-60)}$$

$$a = \frac{1}{3} \cdot \left(3 \cdot q - p^2 \right) \quad \text{(B-61)}$$

$$b = \frac{1}{27} \cdot \left(2 \cdot p^3 - 9 \cdot p \cdot q + 27 \cdot r \right) \quad \text{(B-62)}$$

$$-\alpha_{free} = 2 \cdot \left(\frac{-a}{3} \right)^{0.5} \cdot \cos\left(\frac{\Theta}{3} + \frac{120 \cdot \pi}{180} \right) - \frac{p}{3} \quad \text{(B-63)}$$

$$-\beta_{free} = 2 \cdot \left(\frac{-a}{3} \right)^{0.5} \cdot \cos\left(\frac{\Theta}{3} + \frac{240 \cdot \pi}{180} \right) - \frac{p}{3} \tag{B-64}$$

$$-\gamma_{free} = 2 \cdot \left(\frac{-a}{3} \right)^{0.5} \cdot \cos\left(\frac{\Theta}{3} \right) - \frac{p}{3} \tag{B-65}$$

APPENDIX C
FEATURES OF THE COMPUTER ALGORITHM

A total of four MS Excel® spreadsheets for intravenous and oral administration were set up to describe the pharmacokinetics and pharmacodynamics of prednisolone (PN) and compare prednisolone to a new corticosteroid (NEW). For each route of administration, two spreadsheets are required, for single dosing and multiple steady-state dosing.

The input section includes the dose, time of dosing, and the dosing interval for multiple dosing.

- Dose: The dose is entered in micrograms.
- Time of Dose: The time of dose is entered as the actual clock time of administration in a 24 h interval.
- Dosing Interval: In the case of multiple administrations only, the dosing interval specifies how often the drug is given per day (e.g. a dosing interval of 12 means the drug is administered every 12 h)

The binding parameters describe the binding of prednisolone/ a new drug to plasma proteins. The parameters for prednisolone are for display only and cannot be changed but have to be entered for a new drug.

- Kt: Affinity constant of prednisolone/ the new drug for the binding to transcortin (mg/ng)
- Pt: Concentration of transcortin (ng/mL)
- Ka*Pa: Product of the affinity constant of prednisolone/ the new drug to albumin (mL/ng) and the albumin concentration (ng/mL)

For a new drug, those parameters need to be entered. The parameters for prednisolone are for display only and cannot be changed.

- Q: Liver blood flow of prednisolone/ the new drug (L/h)
- EC50,free: The concentration of the free drug (prednisolone/ new drug) that produces half of the maximum suppressive effect (Emax) (ng/mL)

In the next section of compartmental and noncompartmental parameters, the user has the choice of entering either one set of parameters but not both. The compartmental parameter section includes the dose-independent macroconstants for a three-compartment body model for prednisolone and for a two- or three-compartment body model for a new drug. The parameters for prednisolone cannot be changed.

The noncompartmental section includes different volumes of distribution and intrinsic, and distribution clearances based on noncompartmental analysis. The values for prednisolone are fixed and cannot be changed.

The output section displays different pharmacokinetic and pharmacodynamic parameters. For both, prednisolone and the new drug, it shows the percentage of the CCS based on the total and free drug concentration, respectively. The half-life, volumes of distribution, clearance, and rate constants are entirely based on the free drug concentration.

The graphical section displays the according concentration-time curves of prednisolone and the new drug based on the free, unbound concentration and the total drug concentration. The pharmacodynamic component is shown as the suppression of the endogenous cortisol concentration. These plots are directly linked to the input and output sections and refresh automatically.

APPENDIX D
DERIVATION OF THE EQUATION OF THE FREE FRACTION IN FULL HUMAN PLASMA

The reaction between drug D and protein P to form the drug-protein complex DP in equilibrium can be defined by Equation D-1.

$$[D]+[P]=[DP] \tag{D-1}$$

The equilibrium constant Kd for this reaction can be defined as

$$Kd=\frac{[DP]}{[D]\cdot[P]}. \tag{D-2}$$

Under the assumption of a 1:1 binding, the total drug concentration is the sum of the free, unbound and the bound concentration as shown in Equation D-3.

$$[D_{total}]=[D]+[DP] \tag{D-3}$$

If Equation D-3 is solved for the bound concentration

$$[DP]=[D_{total}]-[D], \tag{D-4}$$

then

$$Kd=\frac{([D_{total}]-[D])}{[D]\cdot[P]}. \tag{D-5}$$

The free fraction of drug D is defined as

$$f_u=\frac{[D]}{[D_{total}]}. \tag{D-6}$$

Dividing the numerator and denominator by the total drug concentration $[D_{total}]$, Equation D-6 becomes

$$Kd = \frac{(1 - f_u)}{(f_u \cdot [P])}. \tag{D-7}$$

It is assumed that only a small percentage of the total protein concentration is bound by drugs. Therefore,

$$[P_{total}] \approx [P]. \tag{D-8}$$

If the protein concentration in Equation D-7 is replaced by the total protein concentration, it changes to

$$Kd = \frac{(1 - f_u)}{(f_u \cdot [P_{total}])}. \tag{D-9}$$

If $f_u^{'}$ is defined as the fraction unbound at another total protein concentration $[P_{total}]^{'}$, then

$$\frac{(1 - f_u)}{(f_u \cdot [P_{total}])} = \frac{(1 - f_u^{'})}{(f_u^{'} \cdot [P_{total}]^{'})}. \tag{D-10}$$

If the values of two different total protein concentrations are

$$[P_{total}] = 5\% \quad \text{and} \quad [P_{total}]^{'} = 100\% \tag{D-11}$$

then

$$f_u^{'} = \frac{f_u}{(20 - 19 \cdot f_u)}. \tag{D-12}$$

If the values of two different total protein concentrations are

$$[P_{total}] = 2.5\% \quad \text{and} \quad [P_{total}]^{'} = 100\% \tag{D-13}$$

then

$$f_u^{'} = \frac{f_u}{(40 - 39 \cdot f_u)}. \tag{D-14}$$

If the values of two different total protein concentrations are

$$\left[P_{total}\right] = 10\% \quad \text{and} \quad \left[P_{total}\right]' = 100\% \tag{D-15}$$

then

$$f_u' = \frac{f_u}{\left(10 - 9 \cdot f_u\right)}. \tag{D-16}$$

If the values of two different total protein concentrations are

$$\left[P_{total}\right] = 15\% \quad \text{and} \quad \left[P_{total}\right]' = 100\% \tag{D-17}$$

then

$$f_u' = \frac{f_u}{\left(6.67 - 5.67 \cdot f_u\right)}. \tag{D-18}$$

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BIOGRAPHICAL SKETCH

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