Solasodine glycosides. Selective cytotoxicity for cancer cells and inhibition of cytotoxicity by rhamnose in mice with sarcoma 180

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Summary

BEC, a standard mixture of solasodine glycosides is effective in vivo against murine sarcoma 180 (S180), whereas the aglycone solasodine at equimolar concentrations is ineffective. The efficacy of BEC against S180 in vivo can be inhibited by rhamnose. Mice which are in their terminal stage with S180 can tolerate and become symptom-free of cancer by single dose administration of BEC at concentrations of BEC three times the LD_{100} for normal mice. These observations suggest that the binding of solasodine glycosides on tumour cells may be mediated through the monosaccharide rhamnose, which forms part of solasonine, solamargine and di-glycosides of solasodine in BEC. Furthermore, these results provide evidence that BEC selectively destroys tumour cells relative to normal cells in vivo.

Keywords: solasodine glycosides; BEC; cytotoxic; sarcoma 180; in vivo

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Introduction

Solasodine glycosides have antineoplastic activity in cell culture [1—3], animals [1,2,4,5], and in humans [1,2,6,7, Cham and Evans, unpublished data]. It has been demonstrated that specific endogenous lectins which are present on the plasma membranes of susceptible cells recognize and bind the sugar moiety of the solasodine glycosides [2,3]. The glycosides are subsequently internalized and cause cell death [2,3].

It was previously shown that a standard mixture of solasodine glycosides (BEC) [8] is effective in vivo against murine S180 [1,2,4]. In such studies, BEC was injected in single and multiple doses upto 4 days after administration of S180 [4]. Rhamnose is not found in mammalian glycoconjugates but forms part of solasonine, solamargine and diglycosides of solasodine in BEC. It was considered that specific receptors for this sugar may be present on cancer cells (absolutely or in greater abundance) relative to normal cells. If these receptors exist, rhamnose would be expected to inhibit the cytotoxic effects of BEC. Here we show that rhamnose inhibits the efficacy of BEC, and that the aglycone solasodine is not effective against murine S180. It was previously shown that mice which were

inoculated intraperitoneally with 5×10^5 S180 tumour cells all died between days 12 and 20 after administration of S180 cells [4]. We now also demonstrate that mice in their terminal stage with S180 can tolerate and become symptom-free of cancer by a large single dose of BEC. The mice tolerate BEC at concentrations which are equivalent to 3 times the LD_{100} of control normal mice.

Materials and Methods

Inhibition of cytotoxicity by rhamnose

Herston White mice with a body weight of approximately 30 g and aged 8—10 weeks served as recipients. Twelve mice were randomly chosen for each experimental group. S180 tumour cells (5×10^5) were inoculated i.p. into mice. This caused a mortality of 100% with a median survival time of 20 days in the control groups. A standard mixture of solasodine glycosides (BEC) was dissolved in dimethylsulfoxide (DMSO) at a concentration of 0.5 g BEC/100 ml DMSO. Similar solutions were made up but also contained 0.3125, 0.625 and 0.9375 g of rhamnose. These solutions were administered i.p. in concentrations of 8 mg/kg animal weight for BEC without and with

5 mg, 10 mg and 15 mg rhamnose/kg animal weight. The first dose was given 0.5 h after administration of the S180 tumour cells. The remaining three doses were given at daily intervals. DMSO and rhamnose had no effect on S180 activity in the absence of BEC.

High dose of solasodine glycosides

Similar conditions were used as previously described [4]. However, in this case, single high doses of BEC 25 mg/kg, 50 mg/kg and 100 mg/kg were administered i.p. 12 days after inoculation of the S180 tumour cells, i.e., 1 day before the animals enter into the terminal stage [4].

Results and Discussion

Figure 1 illustrates that the survival of mice with S180 treated with 4 doses of 8 mg BEC/kg was dependent on given doses of rhamnose. Mice inoculated with S180 cells alone died in 2—3 weeks. When four doses of BEC at 8 mg/kg were given on consecutive days, complete inhibition of S180 activity was achieved and all the animals survived. These results confirm previous studies [4]. The number of survivals was decreased with increasing
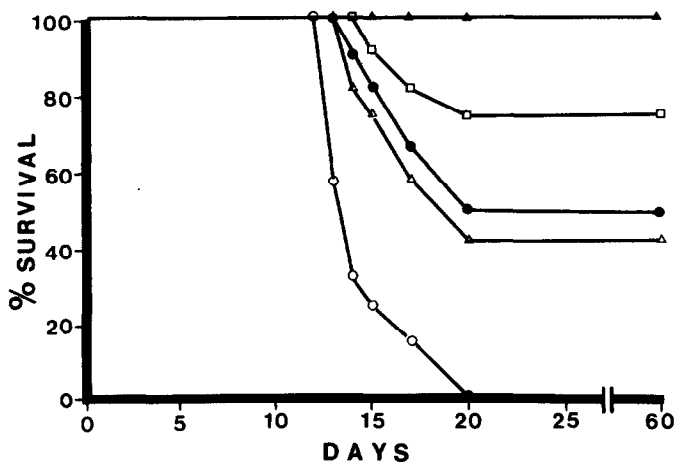

Fig. 1. Effect of solasodine glycosides (BEC) and rhamnose on mouse survival with S180. (o) Untreated S180; (▲) 4 doses BEC 8 mg/kg + rhamnose 15 mg/kg; (●) 4 doses BEC 8 mg/kg + rhamnose 10 mg/kg; (□) 4 doses BEC 8 mg/kg + rhamnose 5 mg/kg; (▲) 4 doses BEC 8 mg/kg.

concentrations of rhamnose. Five milligrams rhamnose/kg decreased the survival to 75%, whereas 10 mg rhamnose/kg decreased the survival to 50% and 15 mg rhamnose/kg decreased the survival to 42%. This indicates that rhamnose may competitively inhibit the efficacy of BEC. Similar concentrations of rhamnose or glucose have no effects on S180 activity in the absence of BEC. These observations suggest that the binding of solasodine glycosides on tumour cells may be mediated through the monosaccharide rhamnose, which forms part of solasonine, solamargine and diglycosides of solasodine in BEC [8].

In all the reported in vivo studies with S180, BEC was injected before the terminal phase [4]. Figure 2 illustrates the effect of single doses of varying concentrations of BEC on the absolute survival of mice which had the S180 tumour for 12 days, i.e., one day before the animals enter into the terminal stage. All animals inoculated with S180 and not treated with BEC died. The survival time is increased with a dose of 25 mg/kg. However, at day 30, all the mice had died. The survival time and number of survivors

were increased with increasing concentrations of BEC, and 17% were symptom-free with a given dose of 50 mg/kg, whereas 42% were symptom-free with a given dose of 100 mg/kg. There are two important observations to note.

The first is that animals which are in their terminal stage can be rendered symptom-free of S180 by BEC therapy. The second is that the animals can tolerate very high doses of BEC. In a previous study, it was shown that the LD_{50} (intraperitoneal) of BEC in mice was 30 mg/kg for single doses and the LD_{100} was 35 mg/kg [4]. Thus, in the present studies, it is shown that if the mice suffered from advanced S180 activity, virtually three times the LD_{100} of BEC for normal mice can be tolerated. This novel and important observation has not been reported with other antineoplastic drugs.

The lack of toxicity may be due to increased plasma or tissue enzymatic activity, resulting in hydrolysis of the sugars from solasodine. Solasodine is relatively non-toxic in mice (100 mg solasodine/kg which is equivalent to approximately 200 mg BEC/kg) does not produce any deaths in mice). But this is unlikely, since
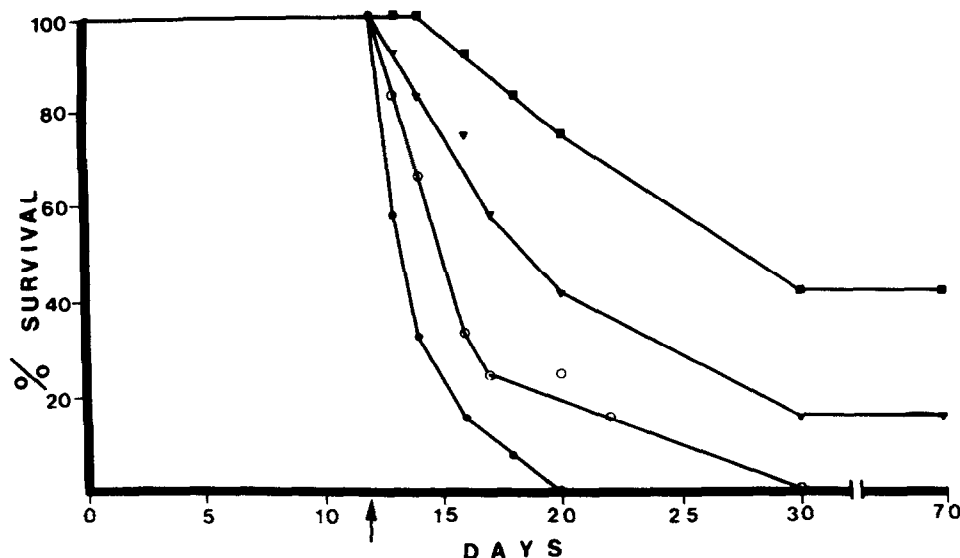

Fig. 2. Effect of high toxic doses of solasodine glycosides (BEC) on mouse survival with S180. Arrow indicates commencement of BEC therapy. (●) Untreated S180; (o) dose BEC 25 mg/kg; (▲) dose BEC 50 mg/kg; (■) dose BEC 100 mg/kg.

solasodine at similar concentrations (100 mg/kg) is not effective in inhibiting S180 activity in mice, and Fig. 2 shows clearly that S180 activity was inhibited by the equivalent concentration of BEC. Alternatively, and a more likely explanation, is that the S180 cells which are in great abundance in the ascitic fluid of the mice 12 days after inoculation of S180 cells, recognize and bind BEC by means of specific receptors, reducing the bioavailability of BEC to normal cells, which in turn reduces the toxicity of BEC. Furthermore, this explanation is supported by the fact that BEC inhibits S180 activity even though the animals are suffering from advanced S180 activity. At this advanced stage, BEC, at concentrations less than 25 mg/kg is not effective in inhibiting S180 activity. These results provide evidence that BEC selectively destroys tumour cells relative to normal cells and the mode of entry of BEC into tumour cells appears to be mediated by the sugar moiety of the solasodine glycosides. These observations are in agreement with in vitro studies [2,3].

Endogenous sugar receptors, also referred to as endogenous lectins, have been biochemically characterized in tumours [9]. Qualitative as well as quantitative differences in histochemical patterns of certain carbohydrate binding proteins in tumours have been reported [10]. It has already been postulated that these sugar-specific receptors may have relevance for improvements in cancer therapy and diagnosis [11,12]. And indeed, it has been shown that tumours exhibit a generally higher capacity to specifically bind labelled (neo) glycoproteins containing various mono- and disaccharides [10], but there are no reports of a rhamnose receptor on such cells.

It is interesting to note that solaplumbinin which is rhamnosyl [3,4] solasodine has also been shown to have anticancer properties in rats [5]. Such observations, together with the current observations and subsequent data on cell culture studies [2,3] strongly suggest that a variety of cancer cell types may have receptors that recognise a rhamnose (or rhamnose-like) residue. The binding of solasodine glycoside to specific receptors on cancer initiate a chain of events, culminating in the internalization of the

glycoside with concomitant delivery of solasodine to the cell. Solasodine, a secondary amine, may then affect the lysosome and other subcellular organelles, resulting in cell death as shown by histological studies [7, Cham and Evans, unpublished data] and cell culture studies [2,3].

It is tempting to speculate that other types of cancers may have specific receptors that recognize certain saccharides which in turn are not recognized and therefore do not bind to normal cells. Therefore, it may be possible to conjugate such a saccharide (or combination of oligosaccharides) to solasodine for the effective and specific treatment of those cancers.

It has been established elsewhere that clinically, BEC is effective and specific for treating topical non-malignant and malignant skin lesions in humans [1,6,7, Cham and Evans, unpublished data]. Although solasodine glyclosides appear to be more specific and effective than other well established antineoplastic drugs such as vinblastine, chlorambucil and cisplatin for treating various forms of cancer cells in cell culture [3], much more work is required to establish whether the solasodine glycosides will have clinical application for internal cancers in humans. Such studies are currently in progress.

Acknowledgements

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