A study of the interaction between *H. pylori* mice passage strains and gastric epithelial cells

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Abstract: *Helicobacter pylori (H. pylori)* infections are very serious health problem that are further worsened by increasing/developing resistance to the current antibiotics. Therefore, new therapeutic agents are needed for *H. pylori* eradication. Use of a CD46 derived peptide (P3) as bactericidal agent against *H. pylori* has shown high activity rate *in vivo* and this study examines the changes in *H. pylori* features in response to effect of P3 treatment.AGS cells were infected with *H. pylori* wild type strain 67:21 and its mice passage strains (P3 treated and untreated strains) and further examined using immunoblotting assay, FACS and Urease activity analysis. Comparatively we found increased level of Urease alpha subunit A (UreA) and alkyl hydroperoxide reductase C (AhpC) proteins for P3 treated strain of *H. pylori* than its wild type or untreated strain after infection of AGS cells. Conclusion These results suggest that there might be a high rate of adherence to host cells for the P3 treated passage strain than untreated or wild type strain. Our findings also indicate that either adhesins are being changed or *H. pylori* interaction to the host cells is affected after P3 treatment.

Keywords: *H. pylori,* CD46 derived peptide (P3), bactericidal, AGS cells.

INTRODUCTION

H. pylori are motile, spiral shaped, 2.5-5.0 µm long and 0.5-1.0 µm wide gram negative bacteria that colonize the human gastric and duodenal mucosa. It is a causative agent of gastric inflammation (gastritis), gastric ulcer and duodenal ulcer which, in chronic conditions lead to gastric cancer (Blaser, 1990; Blaser and Kirschner, 1999; Matsuda *et al*., 2008). Over half of the world's population is colonized by *H. pylori* (Johannes *et al*., 2006). It is the first bacteria to be classified as definite carcinogenic, being the causative agent for gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma (Hemalatha *et al*., 1991). *H. pylori* genome possesses several virulence and colonization factors that are responsible for its persistent infection in the human stomach (Basmarke *et al*., 2011; Oldani *et al*., 2009). These factors include cytotoxin associated gene A (CagA), vacuolatingcytotoxin gene A (VacA), AhpC, urease, various adhesins and the flagella motility system. Bacterial binding is facilitated by various adhesins that are present on the *H. pylori* surface. The outer membrane proteins (OMPs) of *H. pylori* consist of two groups. One is Helicobacter outer membrane porins (Hop) including, blood group antigen binding adhesion (BabA), Sialic acid binding adhesion(SabA), adherence associated lipoprotein A & B (alpA/B), Helicobacter outer membrane protein Z (HopZ), Outer inflammatory protein (OiP) and the second is Hor group (Hop related). Large number of variations have been observed in the expression of OMPs which depend upon the type of host cell and conditions of the environment (Traci *et al*., 2001; Backert *et al*., 2011).The most studied adhesins of *H. pylori* is BabA which binds to the Lewis B antigen and fucosylated blood group O, A and antigen present on the gastric mucosa. SabA binds to the sialyl Lewis A and X antigens (Parreira *et al*., 2011). It has been observed that *H. pylori* infection can modulate receptor expression in the host cells on contact (Backert *et al*., 2011). The signal induction in host cell causes cellular changes and release of cytokines responsible for inflammatory responses. Toxin CagA affects cell proliferation and cell cycle in the AGS cells line (Ding *et al*., 2008). *H. pylori* injects CagA to the host cell via type IV secretion system (TFSS) and is involved in the inductionofcytokine expression in gastricepithelial cells, which is seen as a pronouncedincrease in interleukin (IL8) expression. Toxin VacA induces apoptosis and vacuolation in the epithelial cells (Oldani *et al*., 2009; Traci *et al*., 2001; Burcu *et al*., 2012). The amazing ability of *H. pylori* to survive in the gastric acidic environment is due to urease protein expression. Urease consists of two subunits, alpha and beta i.e., UreA and UreB that make a holoenzyme with cofactor nickel ion (Stingl and De Reuse, 2005). It catalyzes the conversion of Urea into NH_3 and CO_2 and hence increases the pH of surrounding microenvironment for survival (Gisbert and Calvet 2012). Another key enzymatic protein of *H. pylori*, AhpC is important for its growth and colonization. AhpC detoxify hydrogen peroxide (H_2O_2) into water and oxygen and also scavenges organic peroxides and peroxinitrites (Seaver and Imlay, 2001). It has been found that mutant strain in AhpC is very sensitive to oxygen and cannot survive even at the level of < 2% (Matthew *et al.,* 2007).

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A Cell surface protein CD46 is exclusively found in all cells of the human body except RBCs and is recently identified as a receptor for many viruses and bacteria. It regulates complement activation and consists of 4 homologous complement control protein repeats (CCPR-1, -2, -3, and -4). Four major isoforms of CD46 are expressed because of alternative splicing (Dhlman *et al*., 2004; Gill and Atkinson, 2004; Riley-Vargas *et al*., 2004). *H. pylori* bind to CD46 via UreA and AhpC proteins. The mutation study showed that P3 binds to *H. pylori* in the same pattern as CD46. It is further observed that P3 treatment eradicated *H. pylori* from the mice stomach. Both P3 and CD46 inhibited urease activity and affected bacterial survival (Basmarke *et al*., 2011). *H. pylori* infected patients are showing increased resistance to antibiotics. Resistance is found not only to the standard triple therapy (PPI + 2 antibiotics) but also to quadruple therapy and decolonization of bacterium in patient's stomach has been observed after treatment (Gisbert and Calvet, 2012; Iwao *et al*., 2003; Makebongo *et al*., 2009; Irie *et al*., 1997).The increasing resistance of *H. pylori* against antibiotics identifies an urgent need for an alternative therapy capable of eradicating the bacterium. Therefore, the present study is designed to examine the responses of *H. pylori* strain 67:21 to CD46 derived peptide P3 treatment and subsequent interaction with AGS cells.

MATERIALS AND METHODS

Bacteria, cells, mice and antibiotics

The *H. pylori* strains 67:21 were provided by the Department of Genetics, Microbiology and Toxicology, Stockholm University, Stockholm Sweden. Bacteria were grown on Colombia blood agar (CBA) medium supplemented with 8% horse blood (HB) and 8% horse serum (HS) and incubated under humid microaerophilic condition with a 5% O_2, 10% CO_2, and 85% N_2 at 37°C. Human AGS cells were grown inRoswell Park Memorial Institute medium 1640RPMI1640 supplemented with 10% phosphate buffered saline (PBS) incubated at 37°C in a humidified atmosphere of 5% CO_2. A CD46 receptor transgenic mouse strain (hCD46Ge) that expresses CD46 in a pattern closely similar to that in humans was used (Johansson *et al*., 2003and 2005). Animal care and experiments were in accordance with institutional guidelines and approved by institutional ethical board. Mice(n=18) aging 6-8 week old were administered orally with 0.1 ml of *H. pylori* 67:21 at 10^8CFU/ml. At week 8 post-infection, mice were orally treated with 100 µL (20 µmol/L) P3 peptide (treated strain) and or 100 µL of PBS (untreated strain) once a day for 2 weeks. The (n=18) mice were scarified and the bacteria recovered from their stomach were mice passage strains and were used to examine the effect of P3 on bacterium (Basmarke *et al*., 2011).

Immune blotting assay

SDS- PAGE-AGS Cells 900 µl ($1x10^5$ cells) in each well were infected with 100 µl bacteria ($1x10^7$) of all the 3 strains(Wild type strain, P3 treated and Untreated strains) with multiplicity of infection MOI 100:1. The plate was incubated for 6h at 37°C with a 5% CO_2 and washed out unbound bacteria with 1x PBS 3 times. Infected AGS cells were lysed in lysis buffer for 30 min at 4°Cwith constant agitation. Lysate was centrifuged at 10,000xg for 5 min. Then pallet was suspended in loading buffers (62.5mM Tris-HCl pH-6.8, 10% glycerol, 2% SDS, 5% 2-mercaptoetanol, 0.002% bromophenol blue). Samples were cooked for 5min at 95°C, then loaded to gel wells with the help of loading needle. Samples were run first at 80V for 30min through stacking gel then at 150-180V through separating gel (Basmarke *et al*., 2011).

Coomassie staining

To check the protein separation, gel was stained with Coomassie staining solution (0.06% Coomassie R250, 35% isopropanol, and 17.5% acetic acid in water) for 1 min into microwave and then shaked for 10 min. After washing 3 times with water, destaining solution (12% isopropanol and 5% acetic acid in water) was added, heated the gel in microwave for 1min and then kept on shaker at room temperature until destained.

Western Blot

After running the samples through a 12.5% SDS polyacrylamide gel, the proteins were transferred to polyvinyllidene fluoride (PVDF) membrane. The membrane was soaked first in methanol for 30 second, washed for 5 minute with distilled water and then washed for 10 minute with transfer buffer (25mM Tris-base and 192mM glycine). Transfer of proteins from SDS gel to membrane was performed at 22 Volts for 30-60 min using semi-dry transfer system from BIO-RAD. The proteins on membrane were blocked with 5% skimmed milk powder in PBS (SMP/PBS) and incubated for 3 hours at room temperature. After blocking, the membrane was sealed with primary antibody (anti-UreA and anti-AhpC diluted 1:1000 in 1xPBS) in plastic bag and incubation for overnight at 4°C. Next day, washed the membrane 3 x 15 min with Tween buffer, then sealed again with diluted (1:10000 in 1xPBS) secondary antibody (anti-goat for UreA and ant-rabbit for AhpC) with Odyssey stain. Membrane with aluminum cover was incubated for 3 hour and after incubation, proteins were analyzed by machine (ODASSAY Infrared Imager from LI-COR). To ensure that samples were loaded in the same quantity, the original membrane was stripped with stripping buffer of pH 2.0 (1% SDS and 25mM Glycine) for 30 min at room temperature. After incubation, membrane was washed with 1xPBS 3 times, followed by water washing (3 times) and then blocked with 5% SMP/PBS. The membrane reprobed with anti-beta actin antibody (diluted 1:5000 in 1xPBS) and then used secondary antibody (anti-mouse antibody).

Urease activity assay

AGS cells were grown in 24 well plates to 80-90% confluency. *H.pylori* strain 67:21; wild, treated and untreated strains at MOI of 100:1 were added to the wells and labeled as wild, treated and untreated respectively. The plate was incubated for 6 h at 37 °C with a 5% CO_2. After incubation, washed with 1xPBS (3 times) to remove unbound bacteria, then added 200 µl urease working solution (50 mM urea, 50mM potassium phosphate pH. 6.8 and 0.02% phenol red) into each well. The plate was kept at room temperature for 15-20 minutes (min) for urease activity. After 15-20 min the plate samples were read through plate reader at 560 nm.

Fluorescence activated cell scanning (FACS) analysis of H pylori proteins

All three types of strain 67:21 (wild and its passages; P3 treated and untreated) were fixed with cold ethanol (70%) for 5-10 min, then centrifuged at 8000xg and washed with 1xPBS. Samples were stained with an anti- UreA and anti-AhpC antibodies and incubated for 1 hour on ice. After incubation, centrifuged and washed with 500 µl 1xPBS. Then added secondary antibody (Alexa 488 conjugated) at 1:400 dilution and incubated for 30-60 min on ice. After incubation, cells were washed with 500 µl 1xPBS and then analyzed by flow cytometry.

STATISTICAL ANALYSIS

Data were expressed as mean ±SD and the statistical significance was calculated by applying Multiple Comparison Test usingGraphPadPrism version 5; shown as $*P < 0.05$.

RESULTS

Infection of AGS cells with H. pylori strain 67:21 shows high level of UreA and AhpC proteins compared to its wild type strain

In this experiment, UreA (26 kD) and AhpC (21kD) levels were studied after infection of AGS cells with 67:21 strain and its mice passage strains (untreated and treated with P3 peptide). The immunoblotting assay results showed increased level of UreA and AhpC in the P3 treated passage strain than wild type strain. On the other hand, proteins levels for UreA and AhpC in untreated passage strain were similar to wild type strain after infection of AGS cells. The experiment was repeated three times and found the same results for both proteins UreA and AhpC, showing high level for treated passage strain (fig. 1). The anti-actin was used to monitor the quantity of each sample during loading into the gel. Membrane was stripped and reprobed with anti-beta actin antibody and found no difference in their loading quantity (fig. 2). These results showed that either treated strains have expressed more UreA and AhpC in response of P3 treatment to compensate the need of these proteins which were important for bacterial survival or treated strains have gained adoptive features (expression or phase variation of adhesins) to bind host cells more efficiently in order to escape from further unwanted effect of P3 treatment. Samples from all three types of strains (wild and its passages) without AGS cell infection were also blotted as a control (fig. 1). However, we did not see any difference in protein levels of UreA and AhpC.

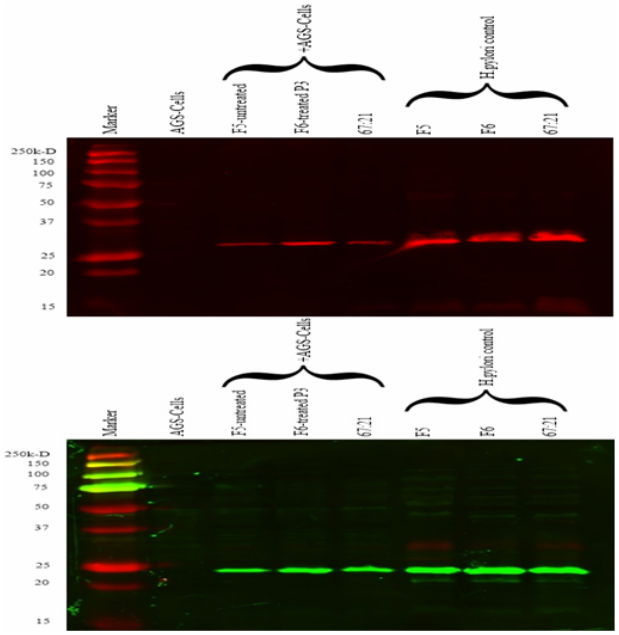

Fig. 1: From left, the first lane is a maker showing proteins size in kilodalton (kD) and the second lane with AGS cells only, used as a control. (A) Membrane is stained with anti-UreA, (B) membrane is stained with anti-AhpC. In both membranes lane 3 has untreated passage (F5) stain, lane 4 treated passage strain(F6) and lane 5 wild(wt) strain. The lanes 6,7and 8 have bacteria samples F5, F6 and wild type respectively.

Expression of UreA and AhpC of H.pylori is not effected with P3 treatment

We observed high level for UreA and AhpC proteins in Immunoblotting assay. Furthermore, FACS analysis was performed to find out if the proteins were expressed in different levels by these strains. All three types of strain 67:21 (wild type, treated passage and untreated mice passages) were stained with UreA and AhpC antibodies followed by staining with Alexa Flour 488 conjugated secondary antibody. FACS results showed same level of UreA and AhpC expression in these strains. These results differed from results obtained in blotting assay because here we did not detect the high level of UreA and AhpC for treated strain as compared to wild type strain. As shown in fig. 3, there is no such significant difference in the expression level of proteins between wild type, treated and untreated strains. Both UreA and AhpC protein levels were found near same for all the three strains. These results show that expression levels of UreA and AhpC are

not altered after P3 treatment; however, expression levels were studied directly without infection of host cells.

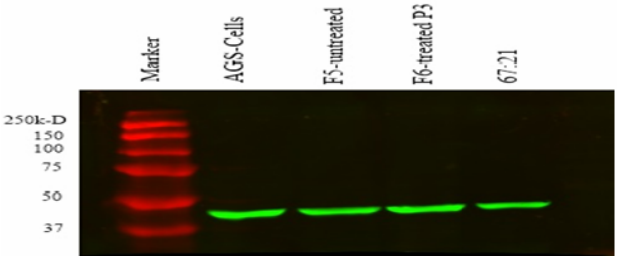

Fig. 2: The membrane stripped and reprobed with anti-actin antibody to monitor the loading quantity of each sample i.e. lane 2 (AGS cells), lane 3 (untreated passage strain-F5), lane 4(treated passage strain-F6) and lane 5 (wt strain).

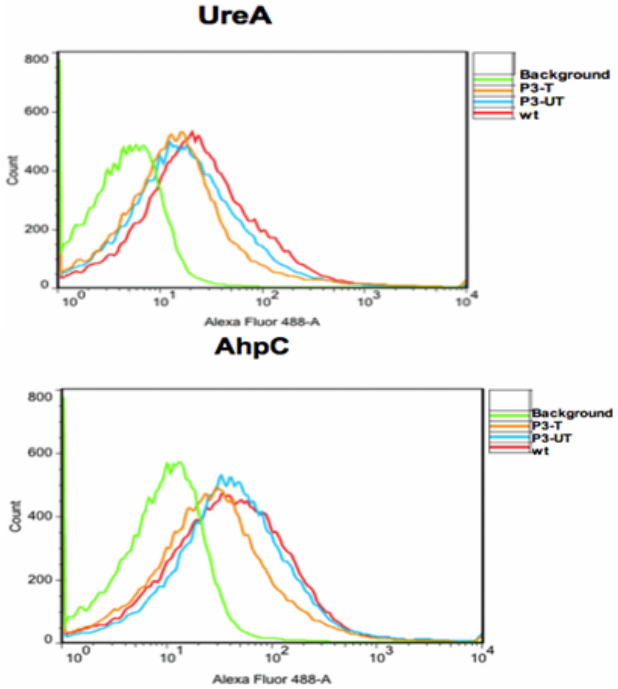

Fig. 3: FACS analysis of UreA and AhpC proteins for *H. pylori*strains 67:21 wild type and their mice passages (treated and untreated). Red linere presents wild (wt), blue untreated (P3-UT) and yellow treated strain (P3-T). (A) Samples of all three stains were stained with antibody to UreA followed by Alexa 488 conjugated anti-rabbitIgG. (B) Samples Strains were stained with anti-AhpC antibody followed by Alexa 488 conjugated anti-rabbit IgG. The green line shows background of antibody staining of these proteins.

Binding efficiency of H.pylori to AGS cells depend upon the type of strain

AGS cells were infected with wild type, treated and untreated strains. After 2 and 6 hour incubation, urease activity for each time point was evaluated. The level of urease activity in each infected AGS cells sample was observed with pH indicator. In the sample solution, the pH of medium was increased by enzymatic action of urease on urea and hence changed the color of phenol red (pH indicator). Table 1 shows results obtained from 6 h incubation experiment. The experiment was repeated in triplicate and found similar results. The absorbance value for treated strain is almost double (abs = 0.843) compared to wild (abs = 0.394) and untreated (abs = 0.462) strain.

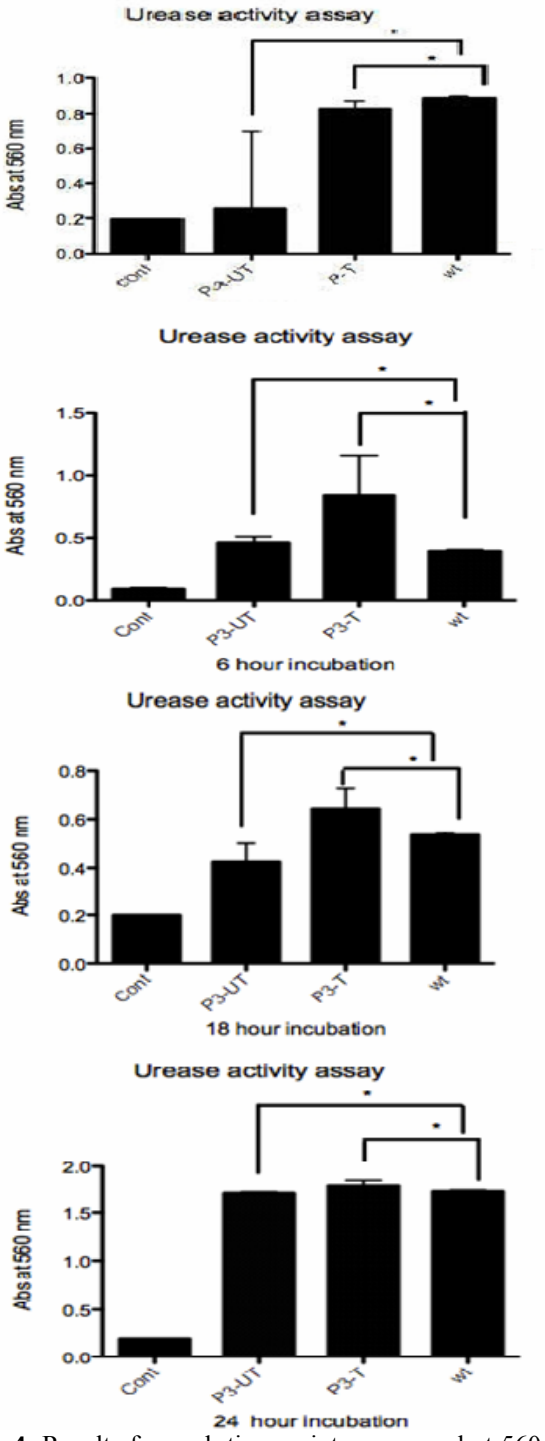

Fig. 4: Results for each time points were read at 560 nm after 15-20 min of adding working buffer solution into well plate of samples. AGS cells (10^7/ml) were infected with strain 67:21 of MOI to 100:1 of each strain (wild and

Table 1: Urease activity assay (6h incubation).

Raw Data (560nm)		Absorbance values are displayed as OD		
H. pylori		P3-treated	Un-treated	AGS-Cells (control
0.409		0.826	0.393	0.093
0.397		0.862	0.502	0.089
0.378		0.84	0.491	0.096
Sum	1.184	2.53	1.39	0.28
Mean	0.394	0.843±0.0097	0.462±0.0031	0.093±0.0074

All bacteria were incubated with AGS cells at MOI. 100:1 for 6h.

passages; P3 treated and untreated). Control AGS cells sample was not infected with *H. pylori* strain.(A) 2 hour Incubation time of infection, (B) 6 hour incubation, (C) 18 hour incubation and(D) 24 hour incubation time of infection. Urease activity levels are mean ± standard deviation based on triplicate samples of each (A, B, C and D) three independent experiments.

In urease activity assay, we observed indirectly the binding of bacteria to the AGS cells. There is an increased level of urease activity with treated strain as compared to wild or untreated strain after 6h incubation at 37°C with 5% CO_2(fig. 4). But this difference in urease activity was not constant at different time periods. In 2h incubation period, the urease activity level of wild strain was little bit higher than passage strains (both treated and untreated strains), but at 6h incubation, results obtained were different (fig. 4B). The level of urease in sample with treated strain was much higher compared to wild type strain. The experiment was repeated in triplicate and found the same results. We also looked for longer period of incubation such as 18h and 24h (fig. 4). The results for 18h showed very small differences in the urease activity between these three types of strain i.e., wild and its passages (passages; treated and untreated). But comparatively, still the level of treated strains was bit high at 18h incubation. On the other hand, the urease activity level on 24hr incubation time was near same in all the three strains i.e. wild, treated and untreated (fig. 3C& D).

DISCUSSION

Failure of triple therapy in *H. pylori* infection is due to the rising incidence of bacterial resistance to antibiotics (Yamamoto *et al*., 2011). Previous studies on antibiotics including amoxicillin are quite encouraging, but the recent reports indicate recolonization of *H. pylori* in a clinical trial (Irie*et al*., 1997). Therefore, scientists are trying to find new bactericidal agents against *H. pylori* infections, but such therapeutic agents need further designing for clinical application. P3 is a 24 amino acid peptide of surface protein CD46 that eliminate bacteria from the mice stomach (Basmarke *et al*., 2011) and the present study was designed to observe P3 effect on *H. pylori* for further characterization as a novel treatment in *H. pylori* infections.

H. pylori virulence factor adhesins mediate its interaction to the host cells. The adhesion proteins of *H. pylori* are under phase variation that enables bacteria to bind specifically to the host receptors (Traci *et al*., 2001). It has been observed that *H. pylori* infection can modulate receptor expression in the host cells on contact (Backert *et al*., 2011). We can assume that different strains i.e. wild and passages will modulate receptor expression in AGS cells differently. In AGS cells infection, our results showed high level of UreA and AhpC proteins with treated passage strain as compared to untreated and wild type strain. But this was not confirmed by FACS results and we were unable to find high or low level of protein expression for all the 3 strains (wild type and its passage strains). Therefore, we suggest that the binding efficiency of strains to the host cells differ from each other. This difference of bacterial binding to AGS cells possibly reflects their differences in adhesins (Dunn *et al*., 1997).

In urease activity assay for 2h incubation, the level of urease in wild type strain was high as compared to passage strain. But in 6h incubation, the level for treated strain was much higher than wild type. Surprisingly, we found small differences in urease activity for all the three strains in 18h incubation, which become almost of the same level after 24h incubation. Thus *H. pylori* might have gone through some kind of adaptation under pressure (P3 treatment) and got stimulus for somehow over expression or switch off/on in genes i.e., adhesins genes for better association with host cells. From these results, we postulate that adhesins are expressed under phase variation to the condition of environment and this is in agreement with the previous reports (Traci *et al*., 2001). This fact raised the possibility that treated strains expressed some genes in the treatment response which enabled bacteria to bind faster as compared to untreated. Our study thus suggests that P3 treatment might induce *H. pylori* for adaptation and rescue from further effect of P3 treatment. Secondly, during incubation, growth of bacteria and specially proliferation of cells might affect the final result in terms of adhesion regarding to wild type and passage strains. Recently, it has been shown that AGS cell cycle is arrested at G1/S phase by *H. pylori* (Ding *et al*., 2008; Belair *et al*., 2011; Mimuro *et al*., 2008) which are dose dependent and it occur when MOI is greater than 100:1. Therefore, we assume that in AGS cell infection

with treated strain, the cell cycle would have not been arrested due to impairment of bacteria. P3 binds to urease and AhpC enzymes of *H. pylori* to render their function (Basmarke*et al*., 2011), thereby providing unfavorable conditions for bacterial growth. The exposure of *H. pylori* to strong hydrochloric acid in stomach will destroy or modify the toxicity of its virulence factors i.e. CagA and VacA. Both CagA and VacA are well studied *H. pylori* pathogenic factors. CagA has been found responsible to arrest the cell cycle while VacA induces apoptosis (Ding *et al*., 2008) and there will be no increase in the number of AGS cells with wild type strain infection. But infection of AGS cells with P3 treated strain will result in increase in the number of cells with time due to proliferation and more bacteria bind to the increased number of AGS cells. Therefore, we found high level of UreA and AhpC in treated passage strain as compared to wild strain and untreated strain. Furthermore, the treated strain would not be able to divide in that ratio as wild strain and the smaller number of bacteria will not be able to arrest the cell proliferation (Ding *et al*., 2008). In the present study, we have observed that P3 treated strain either binds efficiently due to adhesins variation or bacteria bind to high number of AGS cells due to proliferation during incubation.

CONCLUSION

These findings conclude that in response to treatment with bactericidal peptide (P3), *H. pylori* strains tend to adopt some changes which might rescue them from the effect of P3 treatment in terms of quick adhesion to the AGS cells. Possibly, the treated *H. pylori* are no longer affecting the host cell as it is being affected by wild type strain. These findings would be helpful both for further research and clinical application of P3 in *H. pylori* infection.

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REFERENCES

Backert S, Clyne Ms and Tegtmeyer N (2011). Molecular mechanisms of gastric epithelial cell adhesion and injection of CagA by *H. pylori*. *Cell. Commun. Signal.*,**9**: 28.

Basmarke WR, Sjolinder H, Jurkowski W, Elofsson A and Arnqvist A *et al.* (2011).The complement regulator CD46 is bactericidal to *H. pylori* and blocks urease activity. *Gastroenterology,* **14**: 918-928.

Belair C, Baud J, Chabas S, Sharma CM, Vogel J, Staedel C and Darfeuille F (2011). *H. pylori* interfere with an embryonic stem cell miRNA cluster to block cell cycle progression. *Science*, **2**: 7.

Blaser MJ and Kirschner D (1999). Dynamics of *H. pylori* colonization in relation to the host response. *Proc. Natl. Acad. Sci.*, **96**: 8359-8364.

Blaser MJ (1990). *H. pylori* and the pathogenesis of gastroduodenal inflammation. *J. Infect. Dis.*, **161**: 626-633.

Burcu KT, Luisa FJS, Cyeil D, Claudia E and HanR *et al* (2012).Structural insights into *H. pylori* oncoprotein CagA interaction with β1 integrin. *Proc. Natl. Acad. Sci.,* **109**(36): 14640-14645.

Dhlman N, Jacobson RM, Poland and GA (2004). Measles virus receptors: SLAM and CD46. *Rev. Med. Virol.*, **14**: 217-229.

Ding SZ, Smith MF andGoldberg JB (2008). *H. pylori* and mitogen-activated protein kinases regulate the cell cycle, proliferation and apoptosis in gastric epithelial cells. *J. Gastroenterol. Hepatol.*, **23**: e67-e78.

Dunn BE, Cohen H and Blaser MJ (1997). *H. pylori*. *Clin. Microbiol. Rev.*, **10**(4): 720-741.

Gill DB and Atkinson JP (2004). CD46 in *Neisseria* pathogenesis. *Trends. Mol. Med.*, **10**: 459-465.

Gisbert JP and Calvet X (2012). Rifabutin in the treatment of refractory *H. pylori* infection. *Aliment. Pharmacol. Ther.*, **35**: 209-221.

Hemalatha SG, Drumm B and Sherman P (1991). Adherence of *H. pylori* to human gastricepithelial cells *in vitro*. *J. Med. Microbiol.*, **35**(4): 197-202.

Irie Y, Tateda K, Matsumoto T, Miyazaki S and Yamaguchi K (1997). Antibiotic MICs and short time-killing against *H. pylori*: Therapeutic potential of kanamycin. *J. Antimicrob. Chemother.*, **40**: 235-240.

Iwao E, Yokoyama Y, Yamamoto K, Hirayama F andHaga K (2003). *In vitro* and *in vivo* anti *H. pylori* activity of Y-904: A new fluoroquinolone. *J. Infect. Chemother.*, **9**: 165-171.

Johannes GK, Arnoud HM, Van V and Ernst JK (2006). Pathogenesis of *H. Pylori* infection. *Clin. Microbiol. Rev.*, **19**(3): 449-490.

Johansson L, Rytkonen A, Bergman P, Albiger B and Kallstrom H *et al* (2003). CD46 in meningococcal disease. *Science*, **301**: 373-375.

Makobongo MO, Kovachi T, Gancz H, Mor A and Merrell DS (2009). *In vitro* antibacterial activity of acyl-lysyl oligomers against *H. pylori*. *Antimicrob. Agents. Chemother.*, **53**: 4231-4239.

Matsuda K, Yamauchi K and Matsumoto T (2008). Quantitative analysis of the effect of *H. pylori* on the expressions of SOX2, CDX2, MUC2, MUC5AC, MUC6, TFF1, TFF2 and TFF3 mRNAs in human gastric carcinoma cells. *Scand. J. Gastroenterol.*, **43**(1): 25-33.

Matthew A, Croxen PBE and Paul SH (2007). Antisense RNA modulation of alkyl hydroperoxide reductase levels in *H. pylori* correlates with organic peroxide toxicity but not infectivity. *J. Bacteriol.*, **189**: 3359-3368.

Mimuro H, Berg DE and Sasakawa C(2008). Control of epithelial cell structure and developmental fate: Lessons from *H. pylori*. *Bioassays*, **30**: 515-520.

Oldani A, Cormont M, Hofman V, Chiozzi V and Oregioni O *et al* (2009). *H. pylori* counteract the apoptotic action of its VacA toxin by injecting the CagA protein into gastric epithelial cells. *PloS. Pathog*, **5**(10): e1000603.

Parreira P, Magalhães A, Gonçalves IC, Gomes J and Vidal R *et al* (2011). Effect of surface chemistry on bacterial adhesion, viability, and morphology. *J. Biomed. Mater. Res.*,**99**A: 344-353.

Riley-Vargas RC, Gill DB and Kemper C (2004). CD46: expanding beyond complement regulation. *Trends. Immunol.*, **25**: 496-503.

Seaver LC and Imlay JA (2001). Alkylhydroperoxide reductase in the primary scavenger of endogenous hydrogen peroxide. *J. Bacteriol.*, **183**: 7173-7181.

Stingl K and De Reuse H (2005). Staying alive overdosed: How do *H. pylori* control urease activity? *Int. J. Med. Microbiol.*, **295**: 307-315.

Traci L, Testerman, David J, McGee and Mobly HLT (2001). Adherence and Colonization. In: *H. pylori*. Physiology and Genetics, Chapter 34, University of Maryland School of Medicine, 655 W. Baltimore St., Baltimore, MD, 21201.

Yamamoto T, Takano T, Higuchi W, Nishiyama A and Taneike I *et al* (2011). *H. pylori* eradication by sitafloxacin-lansoprazole combination and sitafloxacin pharmacokinetics in Mongolian gerbils and its *in vitro* activity and resistance development. *Antimicrob. Agents. Chemother.*, **55**: 4261-4264.