Journal of Infectious Diseases and Immunity Vol. 3(15), pp. 258-261, 15 December, 2011
Available online at http://www.academicjournals.org/JIDI
DOI: 10.5897/JIDI11.051
ISSN 2141-2375 ©2011 Academic Journals

Full Length Research Paper

Therapeutic effect of nitric oxide and antibiotic against *Salmonella typhimurium*

S. S. Haque

Department of Clinical Biochemistry, Indira Gandhi Institute of Medical Sciences, Patna-14, India.
E-mail: sshaq2002@yahoo.co.in.

Accepted 26 November, 2011

Typhoid fever is an important health menace in India and other developing countries. Human typhoid fever is a severe systemic infection of the reticuloendothelial system caused by the bacterium *Salmonella typhi*. It was estimated that more than 22 million cases of typhoid fever occur in which 200,000 related deaths occur worldwide each year from *S. typhi.* Human typhoid is similar to the infection caused by *Salmonella typhimurium* in mice. In this study antioxidant activity of nitric oxide (NO) and antibiotic were evaluated against the oxidative stress generated by *S. typhimurium* in mice. Infection of mice with bacteria resulted in decrease in the Glutathione peroxidase (GPx) activity by 26.6%, at day 8, as compared to saline treated control and after treatment with formulated drugs, the GPx activity was slightly decreased by 3.11, 4.70, 31.8 and 1.59% as compared with saline treated control.

Key words: Typhoid, glutathione peroxidase, *S. typhimurium*.

INTRODUCTION

Salmonella enterica are frequent causes of disease in humans as well as animals. Invasive salmonellosis is commonly associated with a variety of syndromes that range from gastroenteritis to severe systemic infections. Typhoid fever caused by *Salmonella typhi,* an important global health problem also described by this author (Haque, 2011). The present mode of treatment of typhoid is antibiotics and vaccination, but increasing resistant strain of *S. typhi* has complicated its management, so the search of a newer formulation is required. Nitric oxide (NO) free radicals gas that play significant role in the pathogenesis of typhoid. Nitric oxide is produced in large quantities during host defense and immunologic reactions. The L-arginine nitric oxide pathway in murine macrophages constitutes a primary mechanism of defense against extracellular and intracellular microorganisms (Salvador and Annie, 1993). *S. typhi* will target intestinal epithelial cells (enterocytes), causing the inflammation of other cells in the intestinal Payer's patches and subsequently the mesenteric lymph nodes, spleen, and bone marrow associated with typhoid fever (Ohl et al., 2001). The mechanism by which *S. typhi* attaches to host cell is common among bacteria, which utilizes long, hair-like filaments known as fimbriae that are coated with receptor specific adhesins that recognize and bind to specific types of sites on the surface of target cells (Baulmer et al., 1996). Because these receptors may only be found on certain target cells, great specificity of attachment can be achieved.

For example, *Salmonella* serotypes (e.g. *Salmonella typhimurium*), which each have their own unique antigenic factors, may bind to different cells in the intestine, such as micro fold cells or different macrophages (Ohl et al., 2001). The clinical course of the disease in man varies considerably. It is mostly mild but can also be very severe and life threatening. Though sequels are known to occur in small proportion (2 to 3%) of cases (Archer and Kvenberg, 1985), but these may require expensive and long term treatment (Maki-Ikola and Granfers, 1982). The significance of Salmonellosis does not only stem from the cost incurred for their prevention and control but also because of the severe socio-economic implications. The Food and Drug Administration (FDA) estimated that in 1995, Salmonellosis from food born sources resulted in economic losses of $350 million to 1.5 billion dollars.

Third generation cephalosporin and fluoroquinoles have been found effective in treatment of these cases (Manchanda et al., 2006). However, isolates of *S. typhi* with reduced susceptibility to flouroquinoles (as indicated in the lab by resistance to nalidixic acid) have now appeared in the Indian subcontinent and other regions. These nalidixic acid resistant but ciprofloxacin sensitive strains have increased MIC (minimum inhibitory concentration) for ciprofloxacin although they are still within the current NCLLS (National Committee for Clinical Laboratory Standards) range for susceptibility (0.125 to 0.5 mg/ml) (Rowe et al., 1995; Brown et al., 1996; Jesudason et al., 1996; Chitnis et al., 1999; Kapil et al., 2002).

Glutathione peroxidase is glutathione-dependent seleno peroxidases that catalyze the reduction of hydroperoxides to the respective alchohol.The mechanism for this effect was found to be mediated by oxidation of selenocysteine of the peroxidase to selenocysteine-cysteine bridge (Asahi et al., 1997). The glutathione peroxidase enhanced the formation of nitrite from peroxynitrite and was able to defend against *Salmonella* infection. Nitric oxide may thus interfere with the detoxification of hydroperoxides once formed.

MATERIALS AND METHODS

Dose and dosage

Animals

Swiss albino mice (25 to 30 g) 6 to 8 weeks old were obtained from the central animal house of Hamdard University, New Delhi, India. The animals were kept in poly-propylene cages in an air-conditioned room at 22°/25°C and maintained on a standard laboratory feed (Amrut Laboratory, rat and mice feed, Navmaharashtra Chakan Oil Mills Ltd, Pune) and water *ad libitum*. Animals were allowed to acclimatize for one week before the experiments under controlled light/dark cycle (14/10 h). The studies were conducted according to ethical guidelines of the "Committee for the purpose of control and supervision of Experiments on Animals (CPCSEA)" on the use of animals for scientific research.

Bacteria

In this experiment only *S. typhimurium* (wild) was used. The standard strain of this pathogen was obtained from the National Salmonella Phage Typing Centre, Lady Harding Medical College, New Delhi, India. This bacterial strain was further confirmed by the Department of Microbiology, Majeedia Hospital, New Delhi, India. The drug was administered orally and *S. typhimurium* intraperitoneally.

Animals were divided into six groups. Each group comprised of six animals. The study comprised of the treatment schedule in Table 1. Effects of the drugs in Table 1 on infected mice by *S. typhimurium* were analyzed. Post-treatment of drugs were done at aforementioned dose orally to the experimental animals, the first group was considered as control that receive only saline, second group considered as positive control which was challenged with sub lethal dose of *S. typhimurium* ($0.6xLD_{50}$) along with saline. Third group was challenged with sub lethal dose of *S. typhimurium* and given only full dose of ciprofloxacin. Fourth group was challenged with sub lethal dose of *S. typhimurium* and then mice were treated with full dose of Arginine only. In the fifth and sixth groups, animals were challenged with *S. typhimurium* and then half and one fourth dose of Arginine was administered along with half dose of Ciprofloxacin respectively.

Glutathione peroxidase (GPx) activity

Glutathione reductase (GR) was used for the estimation of GPX activity. The glutathione disulphide produced as a result of GPX activity, which is immediately reduced by GR thereby, maintaining a constant level of reduced glutathione in a reaction system. The assay takes advantage of concomitant oxidation of NADPH by GR, which was measured at 340 nm:

$$H_2O_2 + 2GSH \xrightarrow{GPX} 2H_2O + GSSG$$

$$GSSSG + 2NADPH \xrightarrow{GR} 2GSH + 2NADP^{+} + H^{+}$$

Specific activity of enzyme was measured according to the procedure described by Mohandas et al. (1984). The reaction mixture consisted of 1.44 ml phosphate buffer (0.05 M, pH 7.0), 0.1 ml of EDTA (1 mM), 0.1 ml of sodium azide (1 mM), 0.1 ml of glutathione (1 mM), and 0.1 ml of NADPH (0.2 mM), 0.01 ml of hydrogen peroxide (0.25 mM) and 0.1 ml of PMS (10%w/v) in a final volume of 1.95 ml. The enzyme activity was calculated as nmole NADPH oxidized/min/mg protein by using molar extinction coefficient of $6.22 \times 103\ M^{-1}\ cm^{-1}$.

Statistical analysis

The level of significance between different groups was analyzed by Dunett's t-test after the application of analysis of variance (ANOVA).

RESULTS

Glutathione peroxidase (GPx) activity

GPX activity was significantly assessed in the mice liver concerned with salmonellosis. The mice were challenged with sublethal dose ($0.6xLD_{50}$) of *S. typhimurium*, and then treated with the drugs in Table 1. GPx activity was assessed and the results have been summarized in Figure 1. Infection of mice with bacteria resulted in decrease in the GPx activity by 26.6%, at day 8, as compared to saline treated control. Treatment with drugs, on day 8, the GPx activity was slightly decreased by 3.11, 4.70, 31.8 and 1.59% as compared with control. Formulated drugs maintain normal level of GPx in stress condition which supports our study.

DISCUSSION

Glutathione peroxidase

Similarly, our results suggest that enhanced GPx activity

Table 1. Treatment schedule.

Groups	Treatments
1	Negative control (Normal Saline)
2	Positive control (*S. typhimurium*(0.6xLD_{50})+Saline
3	*S. typhimurium* (0.6xLD_{50})+Ciprofloxacin (400mgper kg b. wt)
4	*S. typhimurium* (0.6xLD_{50}) +Arginine (1000mg perKg b.wt)
5	*S. typhimurium* (0.6xLD_{50}) +Arginine (500mg per kg b. wt) +Ciprofloxacin (200mg per kg b. wt)
6	*S. typhimurium* (0.6xLD_{50})+Arginine(250mgper kg b. wt) +Ciprofloxacin(200 mg per kg b. wt)

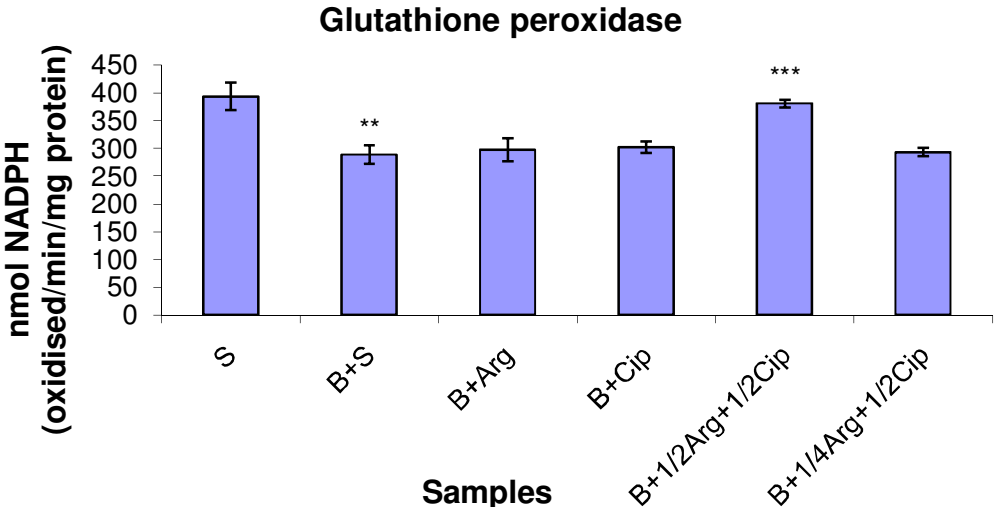

Figure 1. Hepatic GPx activity in mice: drugs were given and study was made on day 8 with arginine, ciprofloxacin and their combination. S=Saline, B+S=*S. typhimurium*+Saline, B+Arg=*S. typhimurium*+1000 mg per kg b. wt L-Arginine, B+Cip=*S. typhimurium*+400 mg per kg b. wt Ciprofloxacin, B+1/2Arg+1/2Cip=*S. typhimurium*+500 mg per kg b. wt Arginine+200 mg per kg b. wt ciprofloxacin, B+1/4Arg+1/2Cip=*S. typhimurium*+250 mg per kg b. wt Arginine+200 mg per kg b. wt Ciprofloxacin. Values are significantly different **p<0.01 and ***p<0.001.

was found in liver in combination (B+1/2 Arg+1/2 Cip) of drugs at day 8 and it was found that maximum increase was seen in L-Arg and combination of drugs (B+1/2 Arg+1/2 Cip) (Figure 1). Results are consistent with study of Farias-Eisner et al. (1996).

It increased the formation of nitrite from peroxynitrite and was able to defend human fibroblast cells against peroxynitrite mediated oxidation. Peroxynitrite is known to inactivate GPx by the oxidation of essential thiol or selenol (Asahi et al., 1997). Therefore, in bacteria infected group the profound decrease in GPX activity could be the result of inactivation of GPX by peroxynitrite. Increase in the GPX activity by the formulated drugs suggested to catabolize peroxynitrite and thus lowers salmonellosis.

Conclusion

This reduction in the oxidative stress enzymes, that is, GPx confirmed the effectiveness of the drug against *S. typhimurium* and so can also be used against typhoid.

REFERENCES

Archer DL, Kvenberg JE (1985). Incidence and cost of foodborne diarrheal disease in the United States. J. Food Prot., 48: 887-894.

Baumler AJ, Tsolis RM, Heffron F (1996). The lpf fimbrial operon mediates adhesion of *Salmonella typhimurium* to murine Peyer's patches. Proc Natl Acad Sci. US A. 9, 279-83.

Chitnis V, Chitnis D, Verma S, Hemvani N (1999). Multidrug-resistant *Salmonella typhi* in India. Lancet, 7;354(9177): 514-515.

Farias-Esner R, Choudhuri G, Aeberhard E, Fukuto JM (1996). The chemistry and tumoricidal activity of nitric oxide/hydrogen peroxide and the implications to cell resistance/susceptibility. J. Biol. Chem., 271: 6144-6151

Haque SS (2011). Biochemical role of nitric oxide precursor and antibiotic against typhoid. J. Microbiol. Antimicrob., 3(8): 217-220.

Jesudason MV, John R, John TJ (1996). The concurrent prevalence of chloramphenicol-sensitive and multi-drug resistant *Salmonella typhi* in chloramphenicol-sensitive and multi-drug resistant *Salmonella typhi* in Vellore, S. India Epidemiol. Infect., 116: 225-227.

Kapil A, Renuka, Das B (2002). Nalidixic acid susceptibility test to

screen ciprofloxacin resistance in *Salmonella typhi*. Indian J. Med. Res., 115: 49-54.
Maki-Ikola U, Granfers U (1982). *Salmonella* triggered reactive arthritis. Lancet, 339: 096-1098.
Manchanda V, Bhalla P (2006). Emergence of non-ceftriaxone-susceptible Neisseria meningitidis in India. J. Clin. Microbiol., 44(11): 4290-4291.
Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller D (1984). Differential distribution of glutathione related enzymes in rabbit kidney: possible implications in analgesic neuropathy. Cancer Res., 44: 5086-5091.
Ohl ME, Miller SI (2001). Salmonella: A model for bacterial pathogenesis. Annu. Rev. Med., 52: 259-274.
Rowe B, Ward LR, Threlfall EJ (1995). Ciprofloxacin-resistant *Salmonella typhi* in the UK. *Lancet*. 11;346 (8985): 1302.
Salvador M, Annie H (1993). The L- arginine-Nitric oxide pathway. The New England J. Med., 329(27): 2002-2012.