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Research Article

EFFECT OF NITRIC OXIDE PRECURSOR AND ANTIBIOTIC ON OXIDATIVE STRESS INDUCED BY SALMONELLA TYPHIMURIUM

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ABSTRACT

Typhoid is systemic infection and in most developing countries remains a major health problem. Due to an increasing resistance to antibiotics and the limited scope of the vaccine the requirement is to explore the efficacy of formulated drugs in the treatment of this disease. In this study we have evaluated the Nitric oxide precursor i.e, L-arginine and Ciprofloxacin in context with the oxidative stress induced.

Key words: Typhoid, Nitric oxide, xanthine oxidase, Catalase

INTRODUCTION

Typhoid fever remains an important cause of illness globally with the annual incidence at 21 million cases, of which 1-4% end fatally [1]. In Asia death due to typhoid was estimated at 90% by the WHO. Typhoid is caused by *Salmonella typhi*. Typhoid is characterized by a high fever, colic pain, inflammation, hepatic injury and diarrhea. *S. typhi* has also been reported to cause hepatic dysfunction and hepatic abscess [2]. The treatment against this disease was antibiotics and vaccination. A major impediment to the effective chemotherapy of typhoid is the ever increasing numbers of resistant strains of *S. typhi*[3-5]. The majorities of the disease occur mainly due to the imbalance between the pro-oxidant and antioxidant homeostatic phenomenon in the body. The condition of proxidant dominates either due to the enhanced generation of free radicals and/or their poor quenching/scavenging into the body. The ability of salmonellae to replicate within the macrophages allows this enteric pathogen to cause this disseminated disease. The bacterial entrance causes the production of superoxide and nitric oxide. Superoxide and nitric oxide react together to form peroxynitrite a strong biological oxidant. Consequently, pathological conditions characterized by oxidative stress can greatly elevate the production of peroxynitrite[6]. The exposure of isolated rat enterocytes to *Salmonella typhimurium* enterotoxin resulted in an increased Xanthine oxidase (XO) activity[7]. Further this author also reported the oxidative stress in mice caused by *S.typhimurium at 14th day of study*[8].

MATERIAL AND METHODS

Dose and Dosage

Animals

Swiss albino mice (25-30g) 6-8 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 (14/10h). 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 *Salmonella typhimurium* (wild) was used. The standard strain of this pathogen was obtained from the National Salmonella Phage Typing Centre, Lady Harding Medical Coll ege, New Delhi, India. This bacterial strain was further confirmed by the Department of Microbiology, Majeedia Hospital, New Delhi, India.

Animals were divided into six groups. Each group comprised of six animals. The study comprised of following treatment schedules.

Effects of above drugs on infected mice by *S. typhimurium* were analyzed. Post-treatment of drugs were done at above dose orally to the experimental animals, 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 fifth and sixth group animals were challenged with *S. typhimurium* and then half and one fourth dose of Arginine was administered alon g with half dose of Ciprofloxacin respectively. On 8th days of post treatment, liver was removed aseptically in sterile condition, homogenate was made and post mitochondrial supernatant was prepared for biochemical estimation.

Table 1:

Groups	Treatments
Group1	Negative control (Normal Saline) (*S. typhimurium*($0.6xLD_{50}$)+Saline
Group2.	Positive control
Group3.	*S. typhimurium* ($0.6xLD_{50}$) + Ciprofloxacin (400mgper kg b. wt)
Group4.	*S. typhimurium* ($0.6xLD_{50}$) +Arginine (1000mg per kg b.wt)
Group5.	*S. typhimurium* ($0.6xLD_{50}$) + Arginine (500mg per kg b. wt) +Ciprofloxacin (200mg per kg b. wt)
Group6.	*S. typhimurium* ($0.6xLD_{50}$) + Arginine (250mgper kg b. wt) +Ciprofloxacin(200 m g per kg b. wt)

Catalase activity

Catalase activity was measured by the method of Claiborne et al, (1985). Briefly, the reaction mixture consisted of 1.95 ml of phosphate buffer (0.1M, pH 7.4), 1ml H_2O_2 (0.09M) and 0.05ml PMS (10%w/v) in final volume of 3ml. Change in absorbance after 3 minutes was recorded at 240nm. Catalase activity was calculated in term of nmol H_2O_2 consumed/min/mg protein.

Xanthine oxidase activity

Xanthine oxidase (XO) is an enzyme that converts xanthine to uric acid. The spectrophotometric method for XO estimation is based on the procedure of Stripe & Corte, (1969) as modified by Ali et al, (2000). Briefly, 0.2 ml of post mitochondrial supernatant was diluted to 1 ml with Tris-buffer (0.5 M, pH 8.1) and incubated for 5 min at 37°C. Reaction was started by adding 0.1ml of 1mM xanthine. The reaction was kept at 37°C for 20 min. The reaction was terminated by the addition of 0.5 ml ice-cold perchloric acid (10%, v/v in distilled water). After 10 min, 2.5 ml of distilled water was added to the precipitated mixture, which was centrifuged at 1,200xg for 10 min. The clear supernatant was decanted and the O.D was read at 290 nm. The results were expressed as µmoles of uric acid formed/mg protein. The activity of xanthine oxidase has been calculated by using a 2mM stock solution of uric acid to prepare standard curve.

Statistical analysis

All data are expressed as means ± standard errors of the means (SEM). The statistical difference was determined by the two-tailed unpaired *t* test. A *P* of <0.05 was considered statistically significant.

RESULTS

Xanthine oxidase (XO) activity

In this study we have evaluated the effect of L-arginine, Ciprofloxacin and their combination on liver damage caused by *S. typhimurium*, XO activity was measured. The mice were challenged with sublethal dose ($0.6xLD_{50}$) of *S. typhimurium* and then treated with above drugs. The results have been summarized in Figures 1 and 2. Infection with bacteria resulted in an increase in XO activity by 11.68% and 35.89% at day 8, 11 as compared with normal control. The treatment of mice with drugs L-arginine, Ciprofloxacin and their combination, at day 8 of infection, caused an increase in the XO activity was reduced to 17.2%, 36.04%, 41.86% and 47.67% respectively as compared with only *S. typhimurium* infected control mice.

Similarly, treatment of mice with drugs L-arginine, ciprofloxacin and their combination, at day 11 of infection, an increase in the XO activity was reduced to 29.24%, 52.83%, 57.54% and 29.24% respectively as compared only with *S. typhimurium* infected control mice.

It is hypothesized that, formulated drugs were able to minimize the damage of liver by reducing the XO activity induced in bacterial infected mice, and maximum reduction was observed at day 11, particularly in Ciprofloxacin and this combination B+1/2 Arg+1/2 Cip. So it can be speculated that combination of drug (B+1/2 Arg+1/2 Cip) might have the capacity to prevent the salmonellosis in murine model.

Catalase (CAT) activity

The effect of L-arginine, Ciprofloxacin and their combination on liver cell damage were assessed, catalase activity was measured. The mice were infected with sublethal dose of *S. typhimurium* ($0.6xLD_{50}$) and then treated with above drugs. Results have been summarized in Figures 3 and 4. The infection with bacteria to control mice resulted in an increase in CAT activity by 20.0% and 22.7% 27.27% at day 8 and 11 respectively..

At day 8, treatment of mice with L-arginine, Ciprofloxacin and their combination, the CAT activity was observed in mice and it was found that reduced activity was increase by 2.7%, 5.5%, 2.7% and 1.38% as compared with control mice. Similarly at day 11, treatment of mice with L-arginine, ciprofloxacin and their combination, significant effect on CAT activity was observed in mice and it was found that reduced activity was increase by 8.82%, 11.76%, 5.88% and 0.00% as compared with control mice. The drugs were able to confer partial enhancement in CAT activity in bacterial infected mice

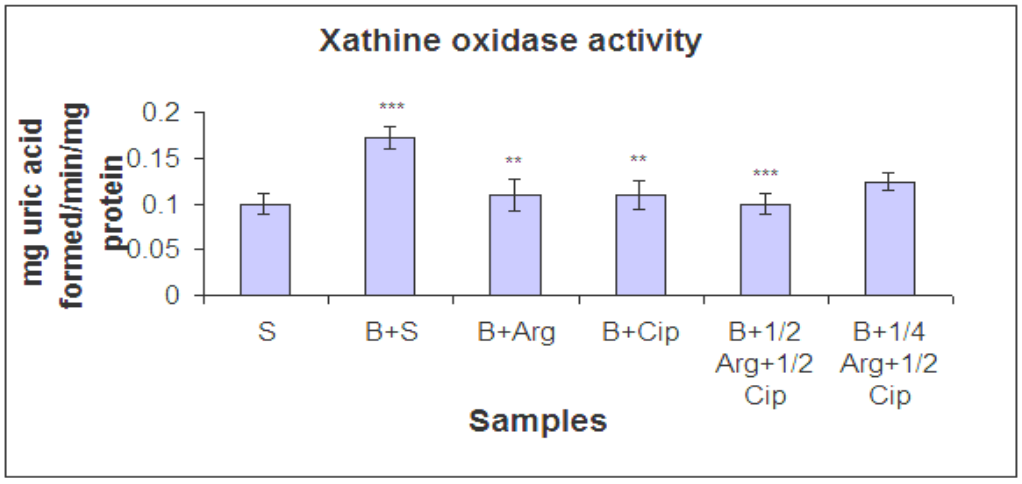

Fig 1: Hepatic XO activities were measured in mice.

Drugs were given and study was made at day 8. S=Saline, B+S=*S. typhimurium*+Saline, B+Arg=*S. typhimurium*+ 1000mg per kg b. wt L-Arginine , B+Cip=*S. typhimurium*+400mg per kg b. wt Ciprofloxacin , B+1/2Arg + 1/2Cip=*S. typhimurium* + 500mg per kg b. wt Arginine +200 mg per kg b. wt ciprofloxacin , B+1/4Arg+1/2Cip=*S. typhimurium*+250mg per kg b. wt Arginine+200mg per kg b. wt Ciprofloxacin.Values are significantly different **p<0.01 and ***p<0.001.

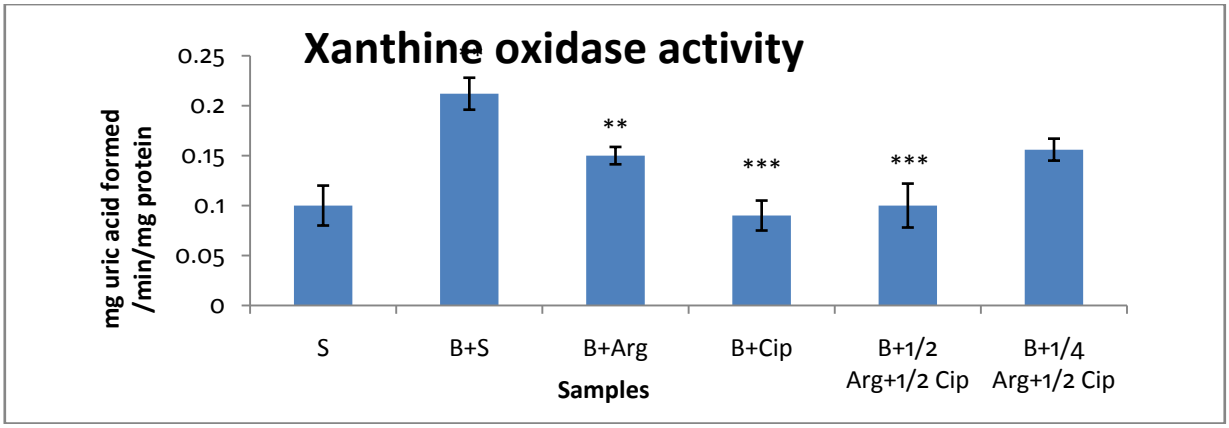

Fig 2: Hepatic XO activities were measured in mice.

Drugs were given and study was made at day 11. S=Saline, B+S=*S. typhimurium*+Saline,B+Arg=*S.typhimurium*+1000mg per kg b. wt L-Arginine B+ Cip=*S. typhimurium*+ 400mg per kg b. wt Ciprofloxacin , B+1/2Arg + 1/2Cip = *S. typhimurium* + 500mg per kg b. wt Arginine + 200 mg per kg b. wt ciprofloxacin, B+1/4Arg+1/2Cip=*S. typhimurium*+250mg per kg b. wt Arginine+200mg per kg b. wt Ciprofloxacin.
Values are significantly different **p<0.01 and ***p<0.001

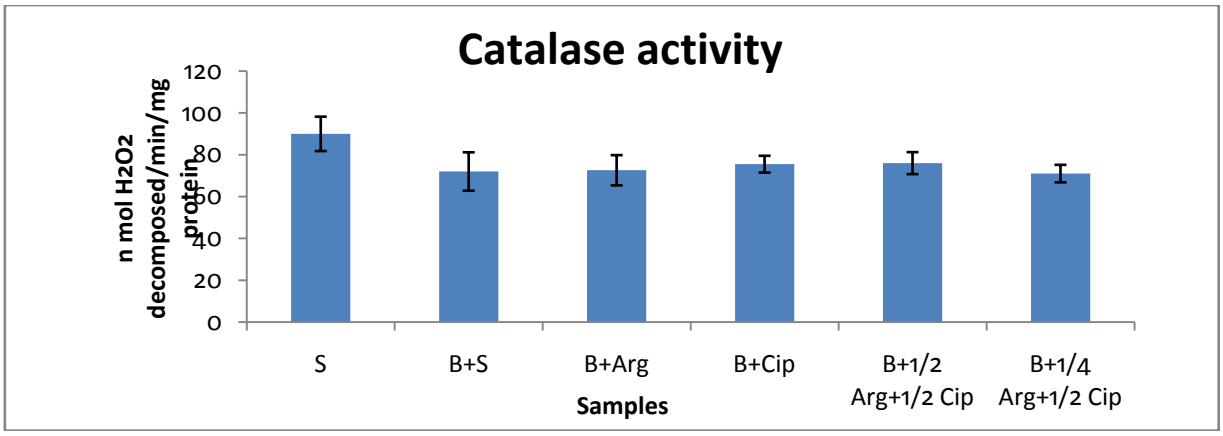

Fig 3: Hepatic catalase activities were measured in mice.

Drugs were given and study was made at day 8. S=Saline, B+S=*S. typhimurium*+Saline, B+Arg=*S. typhimurium*+ 1000mg per kg b. wt L-Arginine , B+Cip=*S. typhimurium* + 400mg per kg b. wt Ciprofloxacin , B+1/2Arg + 1/2Cip= *S. typhimurium* +500mg per kg b. wt Arginine+200 mg per kg b. wt ciprofloxacin, B+1/4Arg+1/2Cip=*S. typhimurium*+250mg per kg b. wt Arginine+200mg per kg b. wt Ciprofloxacin.

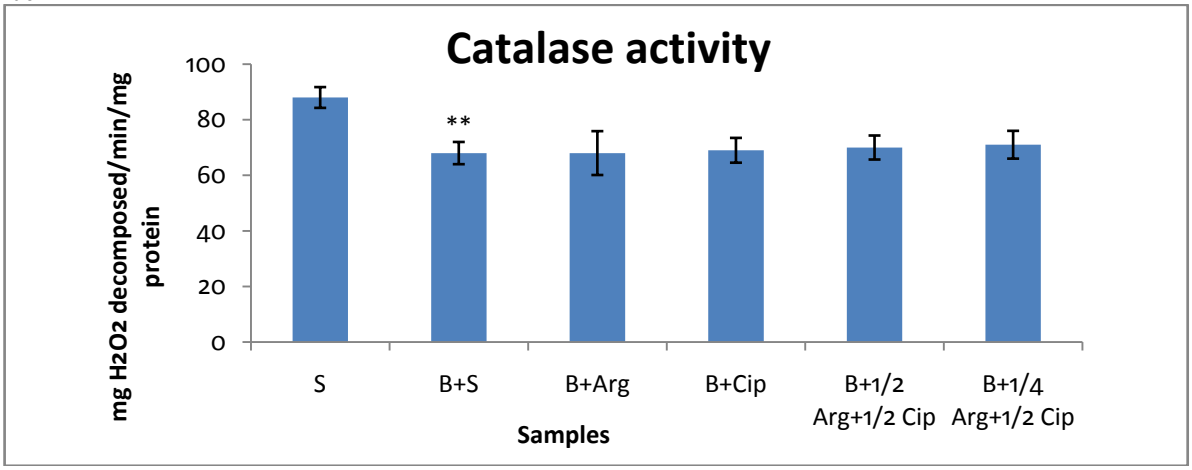

Figure 4: Hepatic catalase activities were measured in mice.

Drugs were given and study was made at day 11. S=Saline, B+S=*S. typhimurium*+Saline, B+Arg=*S. typhimurium*+ 1000mg per kg b. wt L-Arginine, B+Cip=*S. typhimurium*+400mg per kg b. wt Ciprofloxacin , B+1/2Arg +1/2Cip=*S. typhimurium* + 500mg per kg b. wt Arginine + 200 mg per kg b. wt ciprofloxacin ,B+1/4Arg + 1/2Cip = *S. typhimurium* + 250mg per kg b. wt Arginine + 200mg per kg b. wt Ciprofloxacin
Values are significantly different **p<0.01

DISCUSSION

Oxidative stress

Oxidative stress results from the metabolic reaction that uses oxygen, and it has been defined as a disturbance in the equilibrium status of pro-oxidant/anti-oxidant system in intact cells. Oxidative stress has been implicated in human disease by a growing body of facts. However, cells have multiple protective mechanisms against oxidative stress. The main objective of our study was to evaluate the redox status of the cells after treatment with NO precursor,L-arginine and its combination with antibiotic followed by bacterial infection. For this study, we have used liver as the organs of the primary sites for bacterial replication.

Xanthine oxidase

In the present studies, results showed that Uric acid production was decreased in animal treated with drugs (Figures 1 and 2).
The increase of XO activity in *S. typhimurium infected* mice, which in turn enhanced O_2^- production in liver. Similar increase in XO activity in salmonella infected animals was also observed by Umezawa et al, (1997). Peroxynitrite is the main bacteriocidal species in case of *S. typhimurium*[9]. Study reveals that nitrotyrosine, a molecular signatur e that can be associated with peroxynitrite synthesis, does not co-localize with internalized bacteria[10, 11].

Catalase

Catalase is an antioxidant heme-containing enzyme that catalyses the dismutation of hydrogen peroxide into water and oxygen.. Nitric oxide is known to have light affinity for iron in heme proteins[12].It canreversibly bind to ferric iron. This reaction is responsible for inhi bition of catalase by NO[13]. Nitric oxide can inhibit antioxidant metall oenzymes such as catalas[14,15] thereby limiting H_2O_2 disproportionati on.

Mice infected with bacteria showed increase in NO production that results in inactivation of CAT activity but the effect was less pronounced than groups (B+Arg) and (B+Cip). Mice were treated with L-arginine and ciprofloxacin in a combination (B+1/2Arg+1/2Cip), affect CAT activity (Figures 3 and 4). The treatment with L-arginine and ciprofloxacin partially restored the catalase activity especially after 11 days of treatment; however increase in CAT activity was not significant in these cases except (B+Cip)

CONCLUSIONS

The XO activity increased in group B+S, which in turn enhanced O_2^- production in the liver. The $O_2\cdot$ radical has been of profound interest owing to its increased dominance *in vivo* in different disease conditions. Oxidation of hypoxanthine to uric acid with simultaneous generation of O_2^- and H_2O_2 has been observed to play a crucial role during an inflammatory condition and cancer. The formulated drugs exhibit protection by reducing oxidative stress.

REFERENCES

1. Anonymus (2008) WHO, Weekly epidemiological record. 6, 83, 4960. http://www.who.int/wer/2008/wer8306.pdf
2. Soni PN, Hoosen AA, Pillay DG (1994) Hepatic abscess caused by *Salmonella typhi*. A case report and review of the literature. Digestive Diseases Sciences 39, 8, 1694-1696..Goldstein FW, Chumpitaz JC, Guevara JM, Papadopoulous B, Acar JF (1986) Plasmid-mediated resistance to multiple antibiotics in *Salmonella typhi*. Journal of Infectious Diseases 153, 261- 266.
3. Eykyn SJ and Williams H. (1987).Treatment of multiresistant Salmonella typhi with oral ciprofloxacin. *Lancet*. 12; 2, 1407-8.
4. Atoba MA, Charzt FA (2001) In vivo drug resistance during treatment for typhoid fever: a case report. Central African Journal of Medicine 47, 20-21.
5. Rastaldo R, Pagliaro P, Cappello S, Penna C, Mancardi D, Westerhof N, Losano G (2007) Nitric oxide and cardiac function. Life Sciences 81, 779-793.
6. Mehta A, Singh S, Ganguly NK (1999) Effect of *Salmonella typhimurium* enterotoxin (S-LT) on lipid peroxidation and cell viability levels of isolated rat enterocytes. Molecular and Cellular Biochemistry 196, 175-181.
7. Umezawa K, Akaike T, Fujii S, Suga M, Setoguchi K, Ozawa A and Maedia H. (1997). Induction of nitric oxide synthesis and xanthine oxidase and their roles in the antimicrobial mechanism against *Salmonella typhimurium* infection in mice. *Infect Immun*. 65, 2932-2940.
8. Haque SS(2011). Effect of nitric oxide donors and antibiotic againt typhoid. International Research Journal of Microbiology (IRJM)). 2(6) 259-264.
9. Ischiropoulos H, Zhu L and Beckman JS. (1992) Peroxynitrite formation from macrophage-derived nitric oxide . *Arch Biochem Biophys*. 298, 446-451.
10. Crow JP and Ischiropoulos H. (1996). Detection and quantitation of nitrotyrosine residues in proteins *in vivo* marker of peroxynitrite. *Methods Enzymol*. 269, 185-184.
11. Brown GC (1995). Reversible binding and inhibition of catalase by nitric oxide. *Eur J Biochem*. 232, 188-191.
12. Fang FC(1997).Perspectives series, host/pathogen interactions. Mechanisms of nitric oxide-related antimicrobial activity. *J Clin Invest*. 99, 2818-2825.
13. Hoshino M, Ozawa K, Seki H and Ford PC. (1993), Photochemistry of nitric oxide adducts of water soluble iron (iii) porphyrin and Ferrihemoproteins studied by nanosecond laser photolysis. *J Am Chem Soc*. 115, 9568-9575.
14. Cooper CE (1999).Nitric oxide and iron proteins. *Biochem Biophys Acta*. 1411, 290-309.
14. Kim YM, Bergonia HA, Muller C, Pitt BR, Watkins WD and Lancaster Jr. JR (1995). Nitric oxide and intracellular heme. *Adv Pharmacol*. 34, 277-291.
15. Farias-Esner R, Choudhuri G, Aeberhard E and 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.