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Estimation of Nitric Oxide Metabolites and Antibiotic against Typhoid

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Abstract. Background: Typhoid fever (TF) occurs worldwide, primarily in developing countries where sanitary conditions are poor. Nitric oxide (NO) is a gaseous, free radical, molecular species; produced in biological systems. During enzymatic conversion of L-arginine to L-citrulline by NO synthase (NOS) nitric oxide is produced. Earlier studies have suggested that exogenous administration of L - arginine results in increased NO production, indicating that endogenous substrate is insufficient for maximal NO production. By considering these facts, it was thought to see the effect of oral administration of NO donors i.e. L-Arginine along with the low doses of antibiotic (ciprofloxacin). Material and methods: NO estimation was done by the fluorometric method Misko et al, (1993) with slight modification. Results and Discussion: Hepatic nitrite level in mice infected with $0.6xLD_{50}$ of *S. typhimurium* was 8.33%, higher than control animals (treated with saline) at day 8,and in group B+Arg ,B+Cip & B+1/2Arg+1/2Cip were 16.66%, and 12.5% & 10.25% respectively as compared to only *S. typhimurium* infected mice. Conclusion: This increase of nitrite level is may be due to enhanced cytokine expression.

Keywords: Typhoid, Nitric oxide, Antibiotic

1. Introduction

Typhoid or enteric fever is caused by *Salmonella,* are major concern in developing nations especially in South-East Asia, due to unsafe drinking water, contaminated food and lack of basic sanitation measures across large numbers of the population [1]Nitric oxide (NO) is inorganic free radical gas; produced in biological systems. Nitric oxide is produced in large quantities during host defense and immunologic reactions, because it has cytotoxic property and is generated by activated macrophages, it is likely to have a role in nonspecific immunity.The L-arginine nitric oxide pathway in murine macrophages constitutes a primary mechanism of defense against extracellular and intracellular micro organisms [2]. It has been presumed that iNOS expression can provide antimicrobial activity through formation of reactive nitrogen species (RNS) derived from NO [3-5]. For example, peroxynitrite (ONOO), a potent oxidant formed from NO and superoxide radical (O2), is microbicidal for various bacteria, including *Salmonella enterica* serovar *Typhimurium* [6-7], and nitrosothiols, one-electron oxidized derivatives of NO, have potent bacteriostatic activity against serovar Typhimurium [8-9]. In the early 1990's, the emergence of the first three line drugs (chloramphenicol, amoxicillin and cotrimoxazole), resistant strains of S.typhi has necessitated the use of other antimicrobial agents [10-11]. Recently, ciprofloxacin (DNA Gyrase blocker) has successfully been used to treat typhoid fever in adults and children [12-14]. It has been documented that ciprofloxacin penetrates tissue well and that therapeutic concentrations can be achieved in cerebrospinal fluid, even when there is no inflammation of the meninges [15].

This paper will discuss about the contribution of RNS and low doses of ciprofloxacin to *Salmonella* pathogenesis, paying particular attention to our current understanding of the mechanisms by which nitric oxide (NO) helps control *Salmonella* infections and the strategies used by this facultative intracellular pathogen to lessen the cytotoxicity of NO and its nitrosative and oxidative derivatives.

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2. Material and methods

2.1. Dose and Dosage

2.1.1. 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.

2.1.2. 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 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 following treatment schedule. (Table -1)

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

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 along with half dose of Ciprofloxacin respectively.

2.2. Nitric oxide (NO)

This was done by the fluorometric method Misko et al, (1993) with slight modification. This assay is based on the acid catalyzed ring closure of 2, 3-diaminonapthalene (nonfluorescent) with nitrite to form the highly fluorescent product 2, 3-diaminonapthotriazole or 1-(H)-napthotrazole. Briefly the PMS was further subjected to centrifugation at 1,00,000 g for 1hr in order to get the cytosol which was then used for NO estimations. To 0.850ml of phosphate buffer (25Mm, pH-7.4), 50µl of freshly prepared DAN (0.05 mg/ml in 0.62M HCl) was added and mixed immediately. After 10 min incubation at 20^0C, the reaction was terminated with 100µl NaOH (2.8N). The intensity of the fluorescent signal produced by the product is maximized by the addition of base. Formation of 2,3-diaminonaphthotrizole was measured after 5 min at excitation wavelength 385nm and emission wavelength 450nm with slit width of 25% against the standard curve. Luminescence Spectrophotometer (LB50 B, Perkin Elmer, UK) was used to analyze the formation of

fluorescent 2,3-diaminonapthatrizole. The standard curve of nitrite was constructed using different concentrations and was routinely made fresh. NO was expressed as nmole ml^{-1} of cytosol.

3. Results

Murine salmonellosis has long been studied as an alternative to human typhoid, in order to understand pathogenesis of the disease [16, 17]. *Salmonella Typhimurium* is known to initiate infection by penetrating the intestinal epithelium of the small bowel, the area of initial localization being the terminal ileum [18].

3.1. Hepatic nitric oxide (NO) production

Hepatic nitrite levels were used as an indicator of NO production. The nitrite level was measured by fluorometric method, based on acid catalyzed ring closure of 2,3-diaminonepthelene (non flourescent) with nitrite to form highly fluorescent products 2,3-diaminonepthatrizole or 1-(H) nepthatrizole. The results are depicted in Figures 1. Hepatic nitrite level in mice infected with $0.6xLD_{50}$ of *S. typhimurium* was 8.33% higher than control animals (treated with saline) at day 8. Interestingly, hepatic nitrite level in group B+Arg was 16.66% as compared to only *S. typhimurium* infected mice. Although in case of B+1/2Arg+1/2Cip & B+1/4Arg+1/2Cip were 10.25% & 6.41%

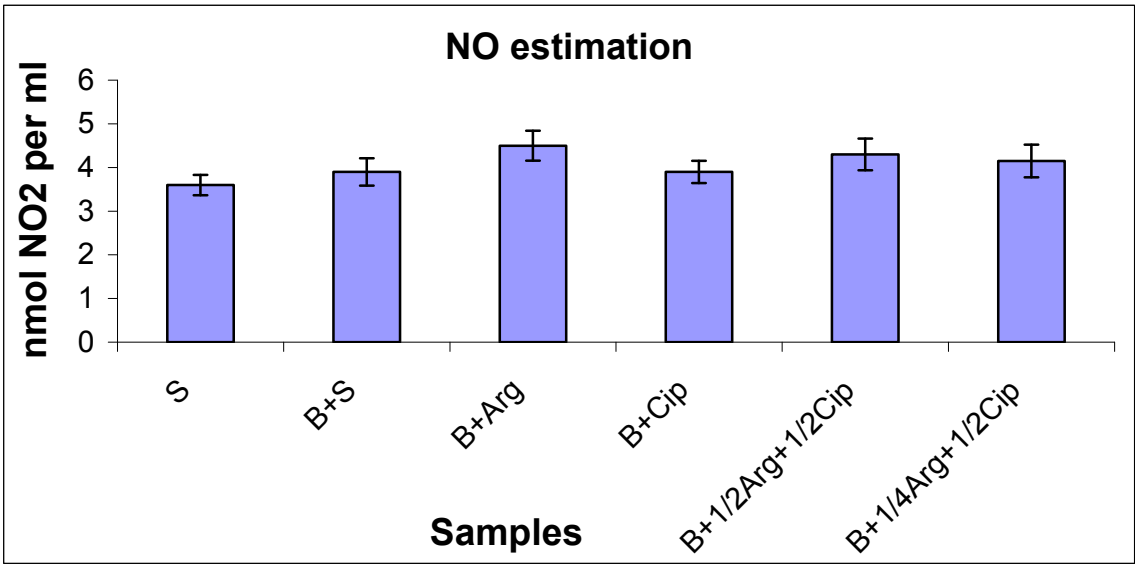

Fig. 1: Nitric oxide production from the reticuloendothelial system: drugs were given and study was made at day 8. S=Saline, B+S=*S. typhimurium*+Saline, B+Arg=*S. typhimurium*+ 1000mg per kg b. wt L-Arginine, B+Cip=*S. typhimurium*+400mg per kg b. wt Ciprofloxacin, B+1/2Arg +1/2Cip=*S. typhimurium*+500mg 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.

4. Discussion

Treatment of L-arg and Ciprofloxacin results increased production of NO following sub lethal challenge with *S. typhimurium* (0.6xLD50) in our study. The NO production following *S. typhimurium* infection is consistent with the study of Umezawa *et al* (1997) [19]. It is now well established that the production of nitric oxide (NO) is the result of increased expression of inducible isoform of nitric oxide synthase (iNOS) which produces NO. Once iNOS is expressed, the level of NO production is dependent, in part, on the concentration of L-arginine, which is the only physiological nitrogen donor for NO production [20,21]. Earlier studies have shown that the physiological levels of L-arginine are rate limiting for NO production [22]. Dietary supplementation with L-arg and L-cit may be an effective way to boost NO production *in vivo [23-24]*. Our results are consistent with these studies. Uninfected groups of mice showed no significant increase in NO production as compared to infected groups. It shows that iNOS is not induced which is necessary to up regulate the NO production (Figures 1).

We evaluate two different combinational doses but found better results at half dose of ciprofloxacin and half dose of L-Arginine, which provide therapeutic induction against typhoid.

5. Conclusion

The hepatic nitrite concentrations may be elevated in case of typhoid. This increase may be due to enhanced cytokine expression, as mentioned by Simpson et al. [25]. If the experimental data are confirmed, modulation of nitric oxide synthesis could represent a new approach to management of typhoid.

6. References

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