Role of Nitric Oxide in Salmonella Infection

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Abstract Nitric oxide (NO) derivative of L-arginine is an important signaling molecule that mediates a variety of essential physiological processes including vasodilation neurotransmission, and host cell defense. Many types of cells produce NO e.g., smooth muscle cell, endothelial cell, and leukocytes. Host defense functions are known for many bacterial and parasitic infections. In the present study we estimated the levels of serum NO in cases of salmonellosis and in controls. The nitric oxide was estimated by cadmium reduction method, Griess reaction. We observed that in controls the level of NO was $(22 \pm 2.06) \mu\text{mol/l}$ and in cases the level was $(137.49 \pm 29.84) \mu\text{mol/l}$. The level of NO was significantly higher than controls ($p < 0.001$). The raised level of NO could be accounted for by host response to the infection. The host rapidly expresses iNOS, which in turn produces an excess amount of NO. Its cytotoxic effect is by its reactive nitrogen oxide derivative e.g., peroxynitrite. Apart from this it also has anti apoptotic functions. In future one can do follow up study of typhoid cases by bacterial culture.

Keywords Nitric oxide · Salmonellosis · Griess reaction · Peroxynitrite

Introduction

Typhoid fever continues unabated in developing countries. Of all the countries Asia accounts for 80% of the cases. In

India typhoid is endemic. A study was conducted by WHO in the year 2003 to compare the disease burden of typhoid fever across Asia. It was seen that incidence of typhoid fever in India was 148.7/100,000 persons/year [1]. In recent years antibiotic resistant strains have been found to be the main cause of infection in India. This is a grave situation as without antibiotic treatment typhoid fever accounts for 10% mortality in those infected. Salmonella typhi usually causes a self limiting acute intestinal inflammation manifesting clinically with diarrhea and vomiting but sometimes it can lead to disseminated potentially serious infection. One of the most characteristic features of salmonella virulence is the ability of the organism to invade mammalian cell. After it is ingested in contaminated food it first invades the epithelial cells of the intestine and then once it has crossed the epithelial barrier it lodges in the macrophages of lamina propria and Peyer's patches. Being an intracellular pathogen, serum antibodies are not the primary defense against the infection. Cell-mediated immunity plays a major role in combating the infection.

Studies have been conducted previously in murine salmonellosis to show the role of nitric oxide in host defense [2]. However no previous studies have been done to see the relation of nitric oxide to human salmonella infection. The present study was undertaken to assess the role of NO in human salmonellosis.

Materials and Methods

The study population included 150 diagnosed cases of typhoid admitted to MSRMC and hospital, Bangalore. The study was conducted with prior permission from the institute's ethical committee.

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Inclusion criteria

- (1) History of fever, diarrhea and vomiting for 3 days.
- (2) Lab investigations- positive WIDAL, positive stool and blood culture.

The admitted patients were enrolled for the study. They recovered and no deaths/complications due to typhoid were reported.

Exclusion Criteria

- (1) History of hypertension, bronchial asthma, diabetes mellitus, chronic renal failure.
- (2) History of intake of drugs like nitrates and steroids.

150 normal and healthy age and sex matched blood donors were taken from the blood bank of MSRMC and Hospital Bangalore as control group.

The serum was collected and nitric oxide was estimated by kinetic cadmium reduction method- Griess reaction [3]. The concentration of nitrite was expressed as $\mu\text{mol/l}$.

Statistical analysis

The data was analyzed using students 't' test. Pearson's formula was used to calculate the coefficient of correlation and students 't' test was applied to see if the correlation was significant.

Results

We found that the level of NO was significantly raised in cases of salmonellosis ($p < 0.001$). See Table 1. No correlation was found between H agglutinin titer and NO levels.

Correlation was found between O agglutinin titer and NO levels. Though it was not statistically significant.

Discussion

It has been shown previously that mononuclear phagocytic cells e.g., macrophages synthesize nitrites and nitrates when they are activated or induced by LPS (lipopolysaccharide)

binding to them. Modlin et al. [4] have shown that LPS binds macrophages through toll-like receptors. Bobby et al. [5] have been able to show that the ultimate mediator for iNOS induction are certain effector proteins SipB, SipC, SipD acting as chaperones along with SopE2 which is a guanine nucleotide exchange factor for Rho GTP ases in both regulation of iNOS expression and Salmonella invasion in murine macrophages. Thus it is likely that the level of iNOS expression and as a result quantum of NO production is determined by both LPS and SopE2. Schafer et al. [6] have shown that Salmonella infection initially i.e., within 3–7 days of infection enhances the cytotoxic activity of NK cells. This is done by increased production of NK cell derived γ -IFN which stimulates NO production by macrophages. Our finding of significantly increased NO in cases could thus be accounted for.

Stamler [7] had shown that NO may react with GSH to form nitrosothiol (GSNO). De groote et al. [8] showed that *S. typhi* exposed to GSNO exhibits cell filamentation. They also demonstrated that this cytotoxicity of GSNO is oxygen independent. They showed that in contrast, peroxyntirite an intermediate of NO is cytotoxic and its action is oxygen dependent. These latter evidences show that NO per se may not be bactericidal and its cytotoxic effect is realized by reactive nitrogen species.

Salmonella species induce apoptosis of host cells via the activation of a cascade of intracellular proteases called caspases. It was recently shown by Alam et al. [9] that during salmonella infection compared to wild type mice, iNOS-deficient mice showed increased number of dendritic cell and significantly higher caspase-3 specific activities. This data confirmed that NO exerts its protective function not only through direct antibacterial action but also by preventing apoptosis and thereby contributing to antimicrobial defense during salmonellosis. The antiapoptotic effect of NO may contribute to host defense by preventing salmonella invasion and dissemination into deeper tissues from the primary septic foci thus localizing the infection.

The findings of our study is supported by Hibbs et al. [10], also who proposed L-arginine: NO pathway as a primary defense mechanism against intracellular microorganisms as well as pathogens like fungi and helminths which are too large to be phagocytosed. In view of these findings they have suggested that administration of L-arginine may result in an enhanced non specific immunity in terms of cytotoxicity and many other modalities.

Two major L-Arginine metabolic pathways studied in leishmaniasis, a model macrophage disease, are particularly relevant due to their role in regulating macrophage effector function. L-Arginine in macrophage can either be catabolized by iNOS to produce NO by arginase for polyamine synthesis depending on the type of extracellular

Table 1 Level of nitric oxide in cases and controls ($\mu\text{mol/l}$)

	Mean $\pm$ S.D. ($\mu\text{mol/l}$)
Controls ($n = 150$)	22 ± 2.06
Cases ($n = 150$)	$137.49 \pm 29.84^*$

* $p < 0.001$

stimuli. So these pathways have been explored for control of infection. Kropf et al. [11] did it by using a synthetic arginase inhibitor. Mukherjee et al. [12] have shown another approach by stimulation of iNOS mediated parasite killing. This inducer is a synthetic peptide having an amino acid sequence of a cysteine protease inhibitor cystatin. Since Salmonellosis has macrophage as the primary effector cell, modulations similar to that done in leishmaniasis could be tried here too.

The beneficial effects of NO as a part of primary immune response that helps the host to survive the infection can be further asserted on doing follow-up studies to demonstrate NO as a prognostic indicator of typhoid. This could be done by both a bacterial culture to check the bacterial load as well as ELISA for salmonella antibody quantification.

In view of these findings, understanding the role of the L-arginine: NO pathway in infection and its modulation may become a valuable therapeutic option in the future.

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