International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

AMP B INDUCED CHANGES OF RAT SERUM NITRITE AND NITRATE LEVELS

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ABSTRACT

The data revealed that the serum of rats receiving either 0.25 and 1.5 mg/kg does of Amp B over 4 and 10 weeks showed, elevated levels of their NO_2^- / NO_3^- (table I & II) confirming that Amp B treatment may enhance the production of more NO end products. For understanding the effects of Amp B on NO pathway end products i.e., NO_2^- / NO_3^-, the author investigated the fate of the above two parameters under Amp B stress and the related data above two parameters under Amp B stress and the related data was included under chapter V (pp 62-67), of this thesis.

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Keywords: *Amp B, Nitrite, Nitrate, Rat Serum.*

INTRODUCTION

NO is a small, membrane permeable free radical synthesized from guanidine nitrogen arginine by the enzyme, NO synthase. It is known that NO is a potent biological mediator molecule produced by variety of cells and organs. Its role as destructor of foreign bodies and acting in the immune response is well known. However, when produced in excessive concentrations, NO can, become toxic and may contribute to cell injury in many diseases [1,2, 3] . The dichotomy of NO is in part due to a broad array or redox species with distinctive properties and reactivities i.e., NO^+,NO and NO^- and it can combine with superoxide anion radical (O_2^-) to yield peroxynitrite ($ONOO^-$) [4] . Under pathological/stress conditions elevated levels of both NO and O_2^-, $ONOO^-$ formed at high yield and contribute for the pathology to $ONOO^-$ is also implicated in the pathogencies $ONOO^-$ directly and rapidly oxidizes sulfhydral groups and initiates lipid peroxidation [5]. Alternatively $ONOO^-$ can nitrate phenolic rings of tryrosine residue of protein, leading to the formation of nitrotyrosine, which usually detected with antinitro tyrosine antibodies [6].

Thus the presence of nitrotyrosine can serve as $ONOO^-$ foot print. The fee radical NO is well suited for its role in intracellular communications; its neutral changes allow free diffusion across biological membranes. In the presence of oxyheamoglobin NO in blood vessels is rapidly converted to nitrate [7].

MATERIALS AND METHODS:

Albino rats of the weight range 150 ± 10 gm were used for the present study. The animals are kept under the constant environmental condition, water and food allowed *ad libitum,* Animals were

divided into eight groups of each seven and were maintained in separate cages.

Treatment of Animals:

The I and III Groups acted as 4 and 10 weeks controls (received equal volumes of sterile water). The II groups of albino rats received 0.25 mg/kg weight of Amp B (i.v) over 4 weeks, the IV groups received 0.25 mg/kg of Amp B over 10 weeks (weekly doses). Simultaneously, the VI groups of rats were administration with 1.5 mg/kg weight of Amp B over 4 weeks(weekly doses) and the VIII groups was treated with 1.5 mg/kg of Amp B over 10 weeks (i.v) (weekly doses). The V and VII group of rats receiving sterile water acted as 4 and 10 weeks experimental control ones. For *invitro* study an Amp B concentrations of 10-500nmol range were suitabily selected.

Statistical Analysis:

For each parameter, the mean of individual observation (for both control and experimental groups were taken into consideration). Statistical significance of the data was analysed through two was ANOVA (analysis of Variance); SNK (Student – Newman - Keuls) test and regression analysis.

RESULT:

The data shown in table I & II shows that Amp B treatment of rat serum has enhanced its nitrite levels *in vivo.* The elevation was found to be less in rat serum receiving 0.25 mg/kg of Amp B over 4 and 10 weeks compared to 1.5 mg/kg/10 wks Amp B treated serum and the elevated nitrite levels were found to be statistically significant ($P<0.001$), over the control. The group of rats receiving 1.5 mg/kg of Amp B over 4 and 10 weeks also exerted elevated levels of their serum nitrite levels (table I) and the changes were found to be statistically significant ($P<0.001$) over the control. (table II) shows that Amp B treated rat serum $N0_3^-$ levels, the group of rats receiving either 0.25 and 1.5 mg/kg of Amp B over 4 and 10 weeks showed elevated levels of their $N0_3^-$ levels and the elevation was found to be statistically significant ($P<0.001$) over the control.

DISCUSSION:

Present study has demonstrated that Amp B in doses tested *in vivo* has enhanced the rat serum $N0_2^-$ / $N0_3^-$ levels and the trends obtained were in agreement with the reports of [8] where they observed elevated macrophage $N0_2^-$ levels in their experiments involving Amp B. The data in table I shows that also confer that a high dose of Amp B employed in the present study (1.5 mg/kg) could contribute for more production of NO end products. Combined this to the experimental data presented in former chapters of this dissertation, where increased NOS activity, ACh content, Ca^{2+} levels and cGMP activity in Amp B treated rat heart, arterioles, ET and SMC cells will support the fact that Amp B administration in rats induces more NO production by way of accelerating various parameters on which NO pathway is known to be dependent and the excess of NO thus formed may be converted to NO_2^- / NO_3^- levels and this probably may be the reason for enhanced levels of rat serum NO_2^- / NO_3^- levels under Amp B stress as observed in this study.

they[9] have reported several cytotoxic effects of NO. NO may react with proteins and nucleic acids. In addition to binding to heme groups e.g., of guanylate cyclase, haemoglobin and cytochrome c oxidase, NO theoretically may react with nucleophilic centers like sulphur, nitrogen, oxygen and aromatic carbons. After diffusion into target cell, NO can inhibit SH-dependent enzymes via S-nitrosylation. NO has been shown to change ion currents through the mitochondrial membrane leading to release of Ca^{2+} in to the cytosol [10,11]. It is long known that activated macrophage inhibit the mitochondrial respiration of target cells [12]. Only the cytochrome c oxidase (complex IV) is inhibited by NO via binding to its heme moiety in a reversible manner [13,14,15]. The nucleus is a further cellular target for NO. NO has been shown to cause G:C A:T transitions and to mediate DNA strand breaks, both suggested to be the results of N-nitrosylation of deoxynucleotides, thus yielding deaminated DNA bases [16] .NO further, was reported to induce oxidative DNA damage in an activated macrophage cell line [17] and to inhibit

enzymes involved in DNA repair. In view of the above mentioned cytotoxic effects of NO, excess of NO generations in Amp B treated rat myocardial system as observed from the present study may cause any of the above cited damages and this inturn may cause myocardial toxicity in rats administered with Amp B.

Despite the manyfold cytotoxic interactions described above, several reports convincingly demonstrate a protective role for NO in oxidative stress. NO inhibits lipid peroxidation by ferrous compounds /H_2O_2 [18], by reactive oxygen intermediates by Fe^{2+} [19], or by azo compounds [20]. Low concentrations of NO have been shown to protect cells from short-term treatment with H_2O_2 [21,22] or with alkylperoxide [23] and to prevent oxidised LDL-or H_2O_2-mediated endothelial cell injury [24,25].

Protective effects of NO have also been reported during cerebral and myocardial ischemia and or reperfusion. These effects of NO are most probably indirect effects of NO as a consequence of its vasodilatory activity to increase the blood flow, its capability to inhibit adhesion of lymphocytes, monocytes and neutrophils to the endothelium and its ability to inhibit platelet aggregation, thus capillary occlusions[26,27] .Vascular NO production protects against ischemic brain injury. NO may be cytoprote.ctive by directly acting as a potent terminator of radical propagation systems or inductor of defense response [9] .Keeping in view of beneficial roles afforded by NO, production of NO in Amp B treated rat myocardial system, may be considered as beneficial in the sense that NO kills pathogens and that Amp B being a potent antifungal agent, the excess of NO formed, in the myocardial systems of rats (as observed from the current study) may kill the pathogens like fungal forms and as reported by [28]. NO production upon Amp B treatment may be one of the mechanisms of action of Amp B.

Table-I: Levels of Nitrite and Nitrate in the Serum of Amp B treated rats. (Values expressed as ng NO_2^- ml of serum).

0.25 mg/kg treated					**1.5 mg/kg treated**			
Rats serum	**Contol**	**4 weeks**	**Control**	**10weeks**	**Contol**	**4 weeks**	**Control**	**10 weeks**
NO_2^- levels								
A V	56.70	63.71	56.20	65.93	57.55	68.13	56.89	73.85
S D	±1.16	±1.22	±1.03	±1.18	±1.014	±1.045	±1.08	±1.106
P C		12.36		17.31		18.38		29.81
t-test		P<0.001		P<0.001		P<0.001		P<0.001
NO_3^- levels								
A V	42.82	45.23	44.52	47.99	44.01	54.52	46.50	71.94
S D	±0.96	±1.01	±0.88	±0.94	±1.16	±1.37	±1.23	±1.28
P C		5.25		7.77		23.88		54.70
t-test		P<0.001		P<0.001		P<0.001		P<0.001

Values expressed as the mean ± S.D of 8 samples.

AV: Average SD: Standard deviation PC: Percent change over control NS: Not Significant * p< 0.001

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