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Research Article

INCREASED SERUM NITRITE AND NITRATE LEVELS IN AMPHOTERICIN-B ADMINISTERED ALBINO RATS

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ABSTRACT

Amphotericin B (Amp-B) usefulness is associated with a number of toxic cellular effects. We investigated the *in-vivo* effects of Amp-B on the nitrite (NO_2^-) and nitrate (NO_3^-) levels in the serum of rats administered with 0.5 or 1.5 mg/kg/wt of Amp-B over 4 or 10 weeks (i.v, weekly doses). The rat serum NO_2^- /NO_3^- levels appeared to be enhanced in the Amp-B administered rats compared to the untreated control group of rats and the changes observed were found to be in a dose and time – dependent manner. Based on the results, it is reported that Amp-B by way of enhancing the production of nitric oxide (NO) may contribute for more production of NO end products like NO_2^-/NO_3^- and these intern may be helpful in reducing the fungal affects, since Amp-B is the drug of choice used against fungal infections in clinical trails.

Keywords: Amphotericin B, Nitric oxide, Nitrite, Nitrate.

INTRODUCTION

The therapeutic use of polyene antibiotic Amphotericin–B (Amp-B) is known to produce a number of side effects such as fever, chills, naucea, vomiting, headache, anoxia, hypoxalamia, renal dysfunction and number of toxicologic side effects in clinical trials as well as in experimental model. L-Arginine in the presence of the enzyme nitric oxide synthase (NOS) is known to produce nitric oxide (NO) and citrulline. NO diffusing into the lumen of blood vessels will be very rapidly converted to nitrate, because of the high concentration of heamoglobin in the red blood cell. NO as soon as it is produced is converted to its end products nitrite (NO_2^-) and nitrate (NO_3^-) [1]. Present study is designed to study the In *vivo* effect of Amp B on rat serum NO_2^- and NO_3^- levels *in vivo.*

MATERIAL AND METHODS

Animals

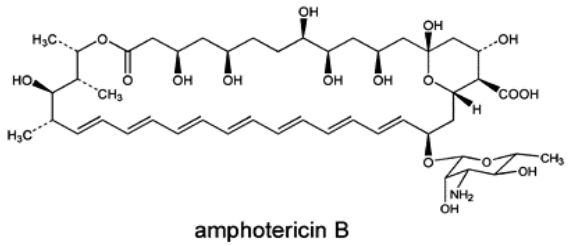

Fig 1: Chemical structure of Amphotericin -B

Albino rats of the weight range 150±10 gm were selected for the present study. The animals were kept under constant temperature of 20±5°C and were fed *Ad labium* on the commercial diet supplied by kamadhenu Agencies, Bangalore. They were divided into six groups of seven each and were maintained in separate cages.

Chemicals

Amp-B ($C_{47}H_{73}NO_{17}$) (Fig 1) was a product from Bristol-Myers Sqibb company, USA. All other chemicals used were of technical grade supplied by SBH, or BDH, India.

Treatment of Animals

Group I and IV rats acted as control over group II rats received 0.5 mg/kg bwt of Amp-B (i.v) in saline over four weeks (weekly doses). Group III rats received 0.5 mg/kg /wt of Amp-B over 10 weeks. Groups V rats were administered with 1.5 mg/kg/wt of Amp-B (i.v) over 4 weeks and group VI rats received 1.5 mg/kg/wt AmpB (i.v) over 10 weeks (Weekly doses) in saline.

After treatment of the animals the control and experimental group of rats were anaesthetized with Ketamine HCL (40mg/kg) and from the individual animals the blood was collected by cardiac puncture, allowed to clotting and the control and experimental samples were subjected for centrifugation at 2500 rpm/15 minutes and the serum was collected into separate cuvettes.

In the control and experimental samples the serum.No_2^-/No_3^- levels were determined following the method of Guarner *et al.*,(1993)[2].

Statistical analysis of the data:

For each parameter, the mean of individual observations (for both control and experimental groups) were taken into considerations and statistical significance of the data was analysed through one way (ANOVA).

RESULTS

The data in table 1 and 2 shows the levels of the control and Amp-B treated rat serum No_2^-/No_3^- levels. In the control rat serum the No_2^- levels were found to be more than No_3^- levels and the Amp-B treated rat serum appeared to show increased levels of its serum No_2^-/No_3^- levels compared to the control over. All the changes were found to be statistically significant ones the control ($P<0.01$). More percent elevation of Amp-B treated rat serum No_2^-/No_3^- levels were observed for rat serum receiving 1.5 mg/kg /wt of Amp-B over 10 weeks (Fig: 1 & 2).

DISCUSSION

Present study has demonstrated that Amp-B in doses tested *in vivo* has enhanced the rat serum No_2^- /No_3^- levels (table:1) and the trends obtained were in agreement with the reports of earlier authors [3] where they observed elevated macrophage No_2^- levels in their experiments involving Amp-B. The data in table 1 & 2 also confirm that a high dose of Amp-B employed in the present study could contribute for more production of NO end products.

Table 1: Impact of Amphotericin-B on rat serum Nitrite (NO_2^{-}) levels in *vivo* (values expressed as ng No_2^- /ml of serum).

Name of the tissue	Control	0.5mg/kg Amp-B		Control	1.5 mg/kg Amp-B	
		4 weeks treated	10 weeks treated		4 weeks treated	10 weeks treated
Rat Serum No_2^-						
AV	62.901	72.600	87.427	60.749	83.039	116.190
SD	± 3.112	±2.590	± 10.250	± 1.945	± 2.339	± 2.167
PC		15.42*	38.99*		36.69*	91.2 *

Each Value is the mean ± SD of 7 Samples.AV:Average,SD:Standard deviation,PC: Percent change over control. * P<0.01

Table 2: Impact of Amphotericin-B on rats serum nitrate(NO_3) levels *in vivo* (Values expressed as ng of NO_3^- /ml of serum).

Name of the tissue	Control	0.5mg/kg Amp-B		Control	1.5 mg/kg Amp-B	
		4 weeks treated	10 weeks treated		4 weeks treated	10 weeks treated
Rat Serum No_3^-						
AV	46.193	61.323	65.014	52.344	75.536	85.829
SD	± 4.029	± 3.334	± 3.577	± 2.363	± 3.734	± 2.843
PC		32.75*	40.75*		44.31*	63.97*

Each Value is the mean ± SD of 7 Samples. AV:Average , SD :Standard deviation ,PC: Percent change over control : * P<0.01

Further the antifungal agent Amp-B appeared to exert dose and time - dependent effect on rat serum No_2^-/No_3- levels. The data supports that the increased rat serum in Amp-B administered rats could be due to more production of NO. Amp-B interacting with NO producing path ways and there by coming the production of more NO is well documented [4-8] and in the present observed trend of result with reports of the above authors. As NO is known to kill pathogens in the body [9], based on the present experimental data it is reported that one of the antifungal mechanisms of Amp-B may involve the production of more NO in conditions of fungal infections.

CONCLUSION

The author attempted to study the *in vivo* effect of Amp-B on rat serum NO_2^-/ NO_3^- levels and the data was presented in Table:1-2; Amp-B administration appeared to enchance the levels of rat serum NO_2^-/ NO_3^- levels for this the reasons were due to diffusion of more NO formed under Amp B stress which thereafter be converted to NO_2^-/ NO_3^- levels.

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