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Research Article

Modulatory Effects of L-Arginine on Methylxanthine-Induced Cardiotoxicity in Rats: A Differential Role for Nitric Oxide (NO)

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ABSTRACT

Methylxanthines are potent bronchodilators used in obstructive airway disease like COPD and bronchial asthma, but the narrow therapeutic index and resultant adverse effect profile have restricted their use. Novel beneficial effects and modes of action are now being proposed for these pharmacoeconomically viable agents. Cardiotoxicity is a prominent adverse effect of methylxanthone and thus we investigated possible mechanisms for such toxicity with an aim to devise ameliorative strategies for counteracting such undesirable effects. In view of the cardioprotective role of nitric oxide (NO) and NO mimetics, the present study investigated the possible modulatory role of L-arginine, a NO precursor, in theophylline induced cardiotoxicity in rats, with a view to exploring strategies for facilitating the safe use of this drug. The methylxanthine, aminophylline induced cardiotoxic effects like increased heart rat, raised mean BP, inverted T-waves and prolonged QTc interval (in ECG). These were accompanied by increased levels of cardiac biomarkers like Troponin-I, CPK-MB, and ADMA. Oxidative stress markers like MDA were elevated whereas, antioxidant defence markers like GSH and SOD were suppressed. Co-administration of L-arginine (with aminophylline) had dose-related effects on cardiac function (heart rate, mean BP, ECG changes) and cardiospecific biomarkers (TnI, CPK-MB, ADMA) - the lower dose being protective whereas the higher dose potentiating some of the cardiac effects and cardiospecific/oxidative stress biomarker levels. The results indicate a biphasic involvement of NO in the cardiotoxic effect of theophylline and suggests possible interactions of NO with reactive oxygen species during such modulations of cardiotoxicity.

Introduction

Cardiotoxicity is associated with the administration of several classes of clinically relevant drugs and can present even after a single administration, or after an overdose. Such drug induced cardiotoxicity attracts considerable attention from basic scientists to clinicians. Adverse cardiac effects are among the leading cause of drug discontinuation and failure of clinical trials, and cardiotoxicity accounted for 45% of all drug withdrawals in recent years and assessing drug-induced cardiotoxicity risk including QT interval prolongation is considered nowadays an integral part of the standard preclinical evaluation of new chemical entities by global regulatory agencies [1-3].

Methylxanthines like theophylline are bronchodilators that have been effectively used therapeutically in cardiorespiratory disorders for nearly a century. Caffeine and theophylline are typical examples of methylxanthines used in therapy and are also found in commonly consumed beverages e.g., tea and coffee. Theophylline (1, 3-dimethylxanthine) is a potent bronchodilator, but the narrow therapeutic index of the drug has limited its use in recent years. Neurotoxicity and cardiotoxicity are among the most prominent adverse effects associate with prolonged use of theophylline which results in morbidity and mortality. Studies reported that theophylline toxicity resulted in metabolic changes like hyperglycemia, hypokalemia, metabolic acidosis, and in severe cases, cardiac arrhythmias, seizures and death [4, 5].

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Theophylline impacts cardiac rate (tachycardia) and rhythm (arrhythmias) at near therapeutic levels, and the positive chronotropic effects are dose dependent. Cardiac arrhythmias have also been reported during theophylline use in an electrocardiographic study [6-10]. Recent studies have indicated the re-emergence of this methylxanthine for the treatment of intractable respiratory conditions, like steroid resistant asthma and COPD, apnea in prematurity etc., and hence there has been a regeneration of interest in methylxanthine research [11-13]. Our recent studies also indicated that theophylline-induced seizures may be due to free radical generation, and interactions of reactive oxygen and reactive nitrogen species were proposed [14].

Nitric oxide (NO) is a unique, multifunctional, gasotransmitter and biological effector involved in the modulation of several cardiovascular processes e.g., vasodilation, cell permeability, platelet function, inflammation, and other vascular processes have advanced our knowledge of the hemodynamic and non-hemodynamic effects. NO is produced by nitric oxide synthases (NOS) and its effects are mediated by cGMP-dependent or cGMP-independent mechanisms. However, growing evidence suggests a crosstalk between the NO signaling and the occurrence of oxidative stress in the onset and progression of vascular diseases, such as hypertension, heart failure, ischaemia, and stroke. For these reasons, NO is considered as an emerging molecular target for developing therapeutic strategies for cardio- and cerebrovascular disease pathologies, and a promising candidate molecule that could find therapeutic application. NO based therapies are known in hypertension, angina, heart failure, and complex mechanisms are proposed [15-17]. However, the role NO in cardiac arrhythmias and particularly such drug-induced cardiotoxicity is less understood. NO is also a free radical that is synthesized by the family of NO synthases from L-arginine and oxygen, yielding L-citrulline as a co-product. Further, dietary arginine is now emerging as a prominent source of NO for therapeutic applications [18, 19]. The present study thus evaluated the possible effects of the NO precursor, L-arginine, on theophylline induced cardiotoxicity and evaluated the possible mechanisms involved therein.

Materials and Methods

I Experimental Animals

Wistar male rats (200-250g) were used in the study. Rats were housed at a constant temperature of 22 ± 2ºC under a 12 h light: 12 h dark cycle. The animals (n=6) per group had free access to food and water throughout the experiment. Animals were maintained as per guidelines in Care and Use of Animals in Scientific Research prepared by Indian National Sciences Academy (INSA), New Delhi. The study protocol had the approval of the Institutional Animal Ethics Committee (IAEC) of VPCI.

II Drugs and Chemicals

The following drugs were used in the study: aminophylline (theophylline with ethylene diamine; water soluble salt of theophylline) and L-arginine (NO precursor). All drugs were procured from Sigma-Aldrich (St. Louis, USA). The drugs were dissolved in distilled water and injected intraperitoneally (IP) in a volume of 1 ml/kg. The ELISA kits used in the study were purchased from Weldon Biotech (New Delhi, India). All other routine chemicals needed for the multiple assays were obtained from SRL Labs (New Delhi, India).

III Experimental Methods

i Measurement of Heart rate, Mean B.P. and ECG

Cardiotoxicity was induced by the administration of aminophylline 50 mg/kg, 100 mg/kg and150 mg/kg IP daily for seven days. After this, the rats were anaesthetised by urethane (1.75 g/kg, IP), kept on a wooden platform, and all four limbs were secured with thread. The trachea was intubated using a polythene cannula and connected to an artificial respirator. The right carotid was cleaned/separated from the accompanying structures in the neck region with the help of a blunt dissector scissor. A bull-dog clamp was placed about 3 cm nearer the heart, and a thread was tied toward the head region. A 24G cannula was inserted into the carotid artery, then connected to the blood pressure transducer and started the BIOPAC -MP- 36 for 5 minutes; care was taken to avoid air bubbles between the cannula and sensor. The hair on the forelimb and hind limb area (abdominal region) was removed with the trimmer and cleaned. The ECG electrodes were fixed, two at forelimbs and two at the hind limb region. After recording cardiovascular parameters, blood samples collected by cardiac puncture and heart were collected from the animals. The blood sample was processed to collect serum and stored at -80ºC for the estimation of various biochemical parameters.

ii Assay for Oxidative Stress Markers

a Malondialdehyde (MDA)

MDA, a marker of lipid peroxidation, was determined as described earlier [20]. Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium lauryl sulfate, 1.5 ml of 20% acetic acid (pH 3.5), 1.5 ml of 0.8% aqueous solution of thiobarbituric acid and 0.2 ml of heart tissue homogenate or PBS. The mixture was made up to 4 ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 5 ml of n-butanol and pyridine (15:1 v/v) and 1 ml of distilled water was added and centrifuged. The organic layer was separated, absorbance was measured at 532 nm using a UV-visible spectrophotometer (UV 5740 SS, ECIL), and MDA content was expressed as nmol/mg protein.

b Reduced Glutathione (GSH)

This assay is based on a reduction of 5, 5'-Dithiobis-(2-nitrobenzoic acid) is by SH groups to form 2-Nitro-5-mercaptobenzoic acid. The nitromercaptobenzoic acid anion has an intense yellow colour that is determined spectrophotometrically at 412 nm [21]. Reagents were mixed, and the colour developed was read immediately at 412 nm on a Shimadzu spectrophotometer against blank.

c Superoxide Dismutase (SOD)

Superoxide dismutase was assayed by the method of Marklund and Marklund, (1974) [22]. Pyrogallol auto-oxidizes rapidly in aqueous solution, the higher the pH, the faster is autoxidation, and several intermediate products are formed. Thus, the solution first becomes yellow brown with a spectrum showing a shoulder between 400-425 nm. Afterwards, the colour begins to turn green, and finally, it turns to yellow. The rate of autoxidation is studied from the linear increase in absorbance at 420 nm, which is seen for several minutes after an induction period of 10 seconds - superoxide anion radical ($O_2^{\bullet-}$)

catalyzes the autoxidation of pyrogallol. Superoxide dismutase inhibits the autoxidation of pyrogallol by dismutase superoxide anion radical.20 mg of heart tissue was homogenized in 2 ml of potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 g at 4ºC in a cooling centrifuge for 20 min. 100 µl of supernatant was added to 3 ml of Tris HCl buffer, pH 8.5 followed by 25 µl of pyrogallol, and then mixed thoroughly. The change in absorbance at 420 nm was recorded at 1 min interval for 3 min.

d 8-Hydroxydeoxyguanosine (8-OHdG)

8-OHdG, a marker for oxidative DNA damage, was determined in blood samples using the commercially available ELISA kit (USCN life science, China). Antibody specific for 8-OHdG was pre-coated into the wells of the microtiter plate. During the reaction, 8-OHdG in the sample competes with a fixed amount of biotin-labeled 8-OHdG for sites on a pre-coated antibody specific to 8-OHdG. Excess conjugate and unbound samples were washed from the plate. Subsequently, avidin conjugated to horse-radish peroxidase was added to each well and incubated for 45 minutes at 37ºC. Finally, TMB substrate solution was added to each well, and the colour change was measured spectrophotometrically at a wavelength of 450nm. The results were expressed in ng/ml.

iii Assay for Cardiac Biomarkers

a Creatinine Phosphokinase (CPK-MB)

CPK-MB, a marker of myocardial ischaemia, was determined in blood samples using ELISA KIT (by USCN Life Science Inc). The microplate well is pre-coated with specific antibody CKMB. The standard or samples were then added to the appropriate microplate wells with a biotin-conjugated antibody specific to CKBB. Next, avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated for a given time. Thereafter, 3, 3', 5, 5'-Tetramethylbenzidine (TMB) substrate solution is added. The enzyme-substrate reaction was stopped by adding the sulfuric acid solution, and the colour change was measured by spectrophotometrically at a wavelength of 450 nm. The concentration of CKMB in the samples was determined by comparing the optical density of the sample to the standard. The results were expressed in ng/ml.

b Brain Natriuretic Peptide (BNP)

BNP, a marker for acute congestive heart failure, was estimated using ELISA kit manufactured by Ray Biotech, Inc. The BNP pre-coated microplate well was used, the samples were added to each well and incubated for 30 minutes at 37ºC.The bound biotinylated BNP peptide interacts with streptavidin-horseradish peroxidase (SA-HRP) which gives colour. The intensity of colour developed is directly proportional to the amount of biotinylated peptide-SA and inversely proportional to BNP peptide in the standard and samples. The colour change is measured spectrophotometrically at a wavelength of 450 nm.

c Troponin-I (TnI)

Troponin-I is one of the reliable biomarkers of cardiac injury. We determined the level of troponin-I by using commercial ELISA kit manufactured by QAYEE-BIO life sciences according to their instruction. Microplate has been pre-coated onto an antibody specific for Tn-I. Samples solution and standards solution is pipette into the wells, and any Tn-I available is bound with the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for Tn-I is added to each well. After washing, avidin conjugated with HRP was added to each well. Followed by a wash to remove any unbound avidin-enzyme reagent. The substrate solution was added to the wells, the reaction is stopped, and the intensity of the colour was measured using ELISA reader.

IV Statistical Analysis

The data were expressed as mean ± SEM and analysed by one-way analysis of variance (ANOVA) followed by post-hoc Tukey's multiple comparison tests. A p value of at least 0.05 was considered as the level of significance in all statistical tests.

Results

I Effects of Aminophylline (Amino) and L-Arginine (L-Arg) on HR, Mean BP, and QTc-Intervals

Administration of aminophylline (amino, 50, 100 and 150 mg/kg) for seven days resulted in increased heart rate and mean blood pressure (BP) in a dose dependent manner, with the effects seen after 150 mg/kg of the drug being most consistent. The changes in ECG also showed similar pattern and was reflected as increased QTc intervals and appearance of inverted T-waves. Hence, the latter dose (150 mg/kg) of the drug was selected for all subsequent drug interaction studies. Pretreatment with the NO precursor, L-arginine (100 mg /kg) attenuated the aminophylline induced increases in the heart rate, mean BP and prolonged QTc intervals (in ECG), and the data of the L-Arg (100) + amino group were comparable with the control group. The incidences of T-wave appearance were also reduced in the ECG. On the other hand, pretreatment with L-arginine (500 mg/kg) did not show such attenuating effects on the HR and mean BP as compared to the amino (150) group. However, the QTc interval was markedly prolonged after co-treatment of amino + L-arginine (500 mg/kg). These results are summarized in (Table 1) and (Figures 1-3).

Table 1: Effects of aminophylline (Amino) and L-arginine (L-Arg) on Heart rate (HR), Mean B.P and QTc interval in rats.

Treatment(mg/kg)	HR (beats/min)	Mean BP (mmHg)	QTc-Intervals(ms)
Control	400.6 ± 9.57	70.8 ± 2.30	411.0 ± 30.86
Amino (150)	550.0 ± 11.41*	95.1 ± 3.21*	512.6 ± 12.46*
L-Arg (100) + Amino	408.3 ± 4.60 #	70.0 ± 3.28	439.2 ± 22.95#
L-Arg (500) + Amino	530.4 ± 2.78	90.7 ± 5.72	614.0 ± 42.73#

All value expressed as mean ± SEM, (n=6/group), *P < 0.05 vs control group, #P< 0.05 vs amino 150 mg/kg treated group.

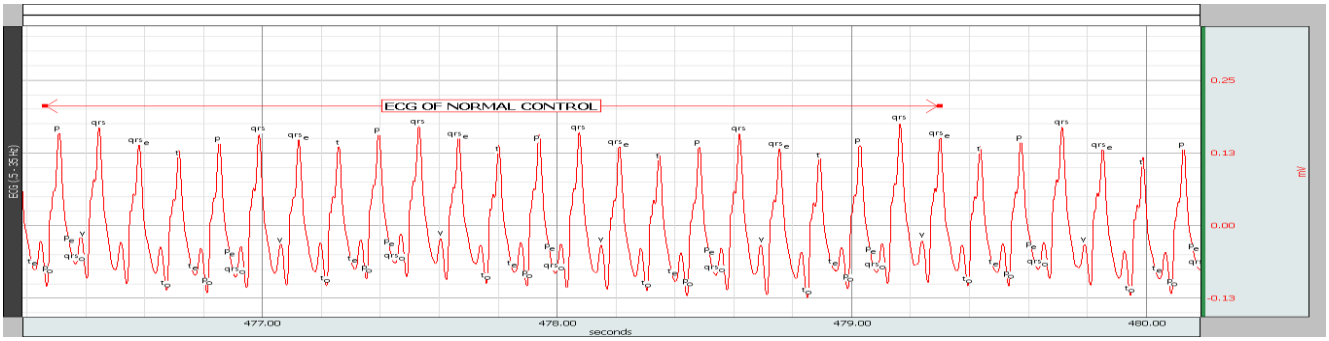

Figure 1: ECG tracing: control group (vehicle), showing normal HR and T-waves.

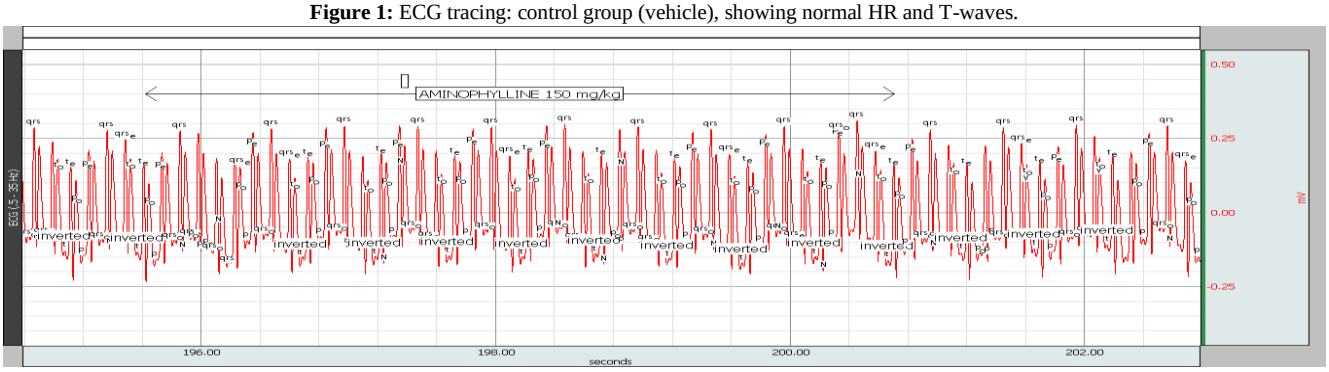

Figure 2: ECG tracing: Aminophylline (150 mg/kg), showing increased HR and inverted T-waves.

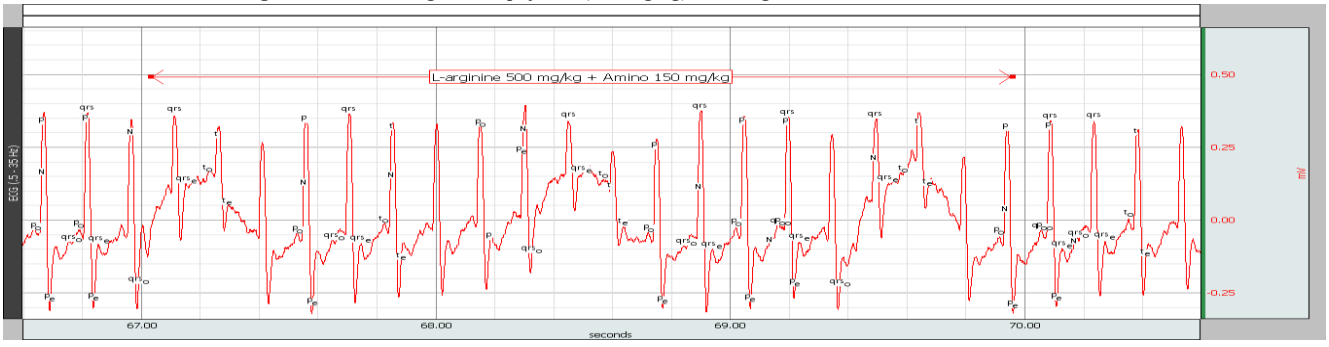

Figure 3: L-arginine 500 mg/kg + Aminophylline 150 mg/kg, showing increased QTc interval and inverted T-waves.

II Effects of Aminophylline (Amino) and L-Arginine (L-Arg) on Oxidative Stress Markers

Aminophylline showed differential effects on the oxidative stress markers studied. There were appreciable increased the malondialdehyde (MDA) levels (by 37%) and 8-OHdGlevels (by 40%); but markedly decreased the reduced glutathione (GSH)and superoxide dismutase (SOD) levels when compared to respective control values ($p < 0.05$). Co-administration of L-arginine 100 mg/kg and amino 150 mg/kg induced differential attenuations in the amino induced changes in oxidative stress markers with significant effects being seen in the GSH levels, which were reverted to near control levels ($p < 0.05$, vs amino 150). On the other hand, co-treatment with L-arginine (500 mg/kg) and aminophylline (150 mg /kg) showed marked aggravations in MDA levels and reductions in SOD levels when compared to the amino 150 group alone ($p < 0.05$). These results are summarized in (Table 2).

Table 2: Effects of Aminophylline (Amino) and L-arginine (L-Arg) on oxidative stress markers in rats.

Treatment(mg/kg)	MDA (nmol/mg protein)	GSH (µmol/mg protein)	SOD (U/mg protein)	8-OHdG(ng/mL)
Control	4.52 ± 0.06	5.2±0.23	3.8±0.10	27.12 ± 2.17
Amino (150)	6.25 ± 0.14	1.7±0.66*	1.8±0.14*	38.23 ± 3.17
L-Arg (100) + Amino	5.17 ± 0.90	5.0 ±0.11#	2.4±1.9	42. 31± 5.12
L-Arg (500) + Amino	12.61 ± 1.01#	1.9±0.91	1.3±0.11#	28.23 ± 8.10

All data expressed as mean ± SEM, (n=6/group), *P< 0.05 vs control group, # P<0.05 vs amino 150 mg/kg.

III Effects of Aminophylline (Amino) and L-Arginine (L-Arg) on Cardiac Biomarkers

The effects of amino 150 mg/kg were evaluated on cardiac biomarkers viz. Troponin-I, CPK-MB, BNP and ADMA. Amino administration for seven days resulted in marked elevations in the levels of Troponin-I, CPK-MB and ADMA when compared to the control group ($P < 0.05$ in all cases). However, no appreciable effects were seen on the BNP levels after amino 150 mg/kg treatment. Co-administration of L-arginine 100 mg/kg and amino 150 mg/kg induced significant attenuations in the

amino 150 induced elevations in cardiac biomarkers and these levels were restored to near normal values of the control group ($p < 0.05$ vs amino 150 group). On the contrary, L-arginine (500 mg/kg) co-administration with amino 150 induced differential increases the levels of cardiac biomarkers, viz. Troponin-I and ADMA, whereas the other biomarkers were not much affected. The results are summarized in (Table 3).

Table 3: Effects of L-arginine (L-Arg) and Aminophylline (Amino) on cardiac biomarkers in rats.

Treatment(mg/kg)	Troponin-I(pg/ml)	CPK- MB (ng/ml)	BNP (pg/ml)	ADMA (µmol /L)
Control	1.5 ± 0.12	4.5 ± 2.17	22.52 ± 0.17	1.18 ± 0.27
Amino (150)	10.2 ± 0.35*	15.5 ± 1.32*	24.21 ± 0.15	5.65 ± 0.31*
L-Arg (100) + Amino	2.4 ± 2.9	5.3 ± 2.12	23.61 ± 0.07	1.28 ± 0.19
L-Arg (500) + Amino	15.1 ± 3.1#	12.2 ± 2.09	23.12 ± 0.05	7.16 ± 0.35#

All data expressed as mean ± SEM, (n=6/ group), *P< 0.05 vs control group; #P<0.05 compared to amino 150 mg/kg group.

Discussion

The results of the present study show that the NO-precursor, L-arginine, has dose-related effects on aminophylline induced cardiotoxicity in rats. The lower doses of L-arginine (100 mg/kg) showed a cardioprotective effect and decreased heart rate, and mean BP induced by amino. Further, Amino induced prolongation of the QTc interval and appearance of inverted T-waves were also attenuated after such L-arginine treatment. These data were supported by low dose L-arginine induced changes in oxidative stress and cardiac biomarkers. Whereas, MDA is a marker of lipid peroxidation, GSH and SOD are both markers of antioxidant defense. Similarly, TnI and CPK-MB are biomarkers of myocardial ischaemia, and asymmetric dimethyl-arginine (ADMA) reflects compromised NO functioning. ADMA competes with arginine for a site on the NOS enzyme and hence causes NO deficiency. Our results suggest that at this lower dose level, L-arginine via generation of NO could exert a protective influence against methylxanthine (aminophylline) cardiotoxicity. Several signaling pathways are reported for NO effects and most recently interactions of NO and reactive oxygen species are reported and reduction in oxidative stress has also been proposed as a mode of action for NO donors in other experimental situations [23].

Methylxanthines like theophylline are known to be metabolized by xanthine oxidase enzyme, which generates free radicals like superoxide. Excess superoxide production could precipitate oxidative stress. Further, superoxide could also combine with other free radicals like nitric oxide (NO) and give rise to peroxynitrite (OONO), which is a reactive nitrogen species capable of aggravating oxidative stress. Major sources of superoxide in the rat heart include the XOR system and NAD(P)H oxidoreductases [24, 25]. Peroxynitrite further leads to lipid peroxidation, and DNA damage [23, 26, 27]. Oxidative stress has also been implicated in ischaemic heart disease and lowering of Amino induced elevations in specific biomarkers by L-arginine (low doses) confirm an antioxidant role for the NO precursor. The fact that L-arginine (at low doses) reversed these effects further suggests a cardioprotective role of the NO precursor. Thus, low dose L-arginine induced cardioprotective effects in our present experiments could have resulted from the oxidative stress ameliorating effects of the NO precursor. Earlier studies from our laboratory showed that oxidative stress was involved in theophylline (aminophylline) induced toxicity [23].

Interestingly and in contrast to what was seen with the lower dose, the higher dose (500 mg/kg) of the NO precursor, L-arginine showed opposite effects on methylxanthine cardiotoxicity, and tended to aggravate some of the cardiovascular changes when co-administered with Amino 150 mg/kg. The augmentation of cardiac parameters viz. HR, mean BP, inverted T-waves and prolonged QTc (in the ECG), with raised cardiac biomarkers like TnI, CPK-MB and ADMA, were greater than those seen with aminophylline per se. These cardio-specific responses were also accompanied by elevated levels of MDA (a marker for oxidative stress) and lowered levels of SOD (antioxidant defense), and these alterations were greater in magnitude as compared to those seen after Amino 150 mg/kg, per se. Collectively, they were suggestive of a potentiating effect of the NO precursor on methylxanthine cardiotoxicity. NO could be cardioprotective or cardiotoxic depending on the signaling pathway used and persistent high levels of NO could be toxic to the cardiomyocyte via peroxynitrite (OONO) production [15].

The toxicity of NO is markedly enhanced by its reaction with superoxide to form peroxynitrite [28]. Earlier studies also reported that the administration of peroxynitrite ($OONO^{\cdot}$) into the rat heart impaired cardiac contractile function by decreasing cardiac efficiency [29]. Moreover, the endogenous formation of $OONO^{\cdot}$ in the heart contributes to myocardial stunning in ischaemia/reperfusion injury and to the spontaneous loss of cardiac function in the isolated working rat heart [30-33]. L-arginine, a precursor of NO, co-administered with aminophylline, could have resulted in high concentrations of NO which interacted with superoxide anion radicals to yield peroxynitrite (OONO). It is noteworthy to mention that interactions of methylxanthines with NO have also been reported in other disease models [34, 35]. Taken together, our study suggests that the NO precursor, L-arginine when given in low doses could protect against methylxathine induced cardiotoxicity, but higher doses could have deleterious cardiovascular effects. These findings are of translational value as methylxanthines are re-emerging as viable adjuncts to conventional therapy in respiratory diseases like COPD and bronchial asthma and controlled dietary arginine intake could help in rationalizing pharmacotherapy in these conditions by preventing unwanted adverse effects.

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Ethical Approval

The study protocol had the approval of the Institutional Animal Ethics Committee (IAEC) of VPCI.

Conflicts of Interest

None.

REFERENCES

1. Varga ZV, Ferdinandy P, Liaudet L, Pacher P (2015) Drug-induced mitochondrial dysfunction and cardiotoxicity. *Am J Physiol Heart Circ Physiol* 309: H1453-H1467. [Crossref]
2. Mladěnka P, Applová L, Patočka J, Costa VM, Remiao F et al. (2018) Comprehensive review of cardiovascular toxicity of drugs and related agents. *Med Res Rev* 38: 1332-1403. [Crossref]
3. McGowan JV, Chung R, Maulik A, Piotrowska I, Walker JM et al. (2017) Anthracycline Chemotherapy and Cardiotoxicity. *Cardiovasc Drugs Ther* 31: 63-75. [Crossref]
4. Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* 51: 83-133. [Crossref]
5. Helliwel M, Berry D (1979) Theophylline poisoning in adults. *Br Med J* 2: 1114. [Crossref]
6. Eriksson Jr CE, Writer SL, Vestal RE (1987) Theophylline-induced alterations in cardiac electrophysiology in patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis* 135: 322-326. [Crossref]
7. Urthaler F, Jamies TN (1976) Both direct and neutrally mediated components of the chronotropic actions of aminophylline. *Chest* 70: 24-32. [Crossref]
8. Peck CC, Lenert LL, Baker J, Dayan E (1983) Theophylline induce bronchodilator lag serum theophylline levels under non steady state condition. *Clin Pharma Ther* 33: 259.
9. Vestal RE, Eiriksson Jr CE, Musser B, Ozaki LK, Halter JB (1983) Effect of intravenous aminophylline on plasma levels of catecholamine and related cardiovascular and metabolic responses in man. *Circulation* 67: 162-171. [Crossref]
10. Sessler CN, Cohen MD (1990) Cardiac arrhythmias during theophylline toxicity. A prospective continuous electrocardiographic study. *Chest* 98: 672-678. [Crossref]
11. Makino S, Fueki M, Fueki N (2004) Efficacy and safety of methylxanthines in the treatment of asthma. *Allergol Internat* 53: 13-22.
12. To Y, Ito K, Kizawa Y, Failla M, Ito M et al. (2010) Targeting phosphoinositide-3-kinase-delta with theophylline reverses corticosteroid insensitivity in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 182: 897-904. [Crossref]
13. Onatibia Astibia A, Martínez Pinilla E, Franco R (2016) The potential of methylxanthine-based therapies in pediatric respiratory tract diseases. *Resp Med* 112: 1-9. [Crossref]
14. Gulati K, Ray A, Pal G, Vijayan VK (2005) Possible role of free radicals in theophylline-induced seizures in mice. *Pharmacol Biochem Behav* 82: 241-245. [Crossref]
15. Strijdom H, Chamane N, Lochner A (2009) Nitric oxide in the cardiovascular system: a simple molecule with complex actions. *Cardiovasc J Afr* 20: 303-310. [Crossref]
16. Divakaran S, Loscalzo J (2017) The Role of Nitroglycerin and Other Nitrogen Oxides in Cardiovascular Therapeutics. *J Am Coll Cardiol* 70: 2393-2410. [Crossref]
17. Forte M, Conti V, Damato A, Ambrosio M, Puca AA et al. (2016) Targeting Nitric Oxide with Natural Derived Compounds as a Therapeutic Strategy in Vascular Diseases. *Oxid Med Cell Longev* 2016: 7364138. [Crossref]
18. Förstermann U, Sessa WC (2012) Nitric oxide synthases: regulation and function. *Eur Heart J* 33: 829-837. [Crossref]
19. Levine AB, Punihaole D, Levine TB (2012) Characterization of the role of nitric oxide and its clinical applications. *Cardiology* 122: 55-68. [Crossref]
20. Joshi JC, Ray A, Gulati K (2014) Differential modulatory effects of morphine on acute and chronic stress-induced neurobehavioral and cellular markers in rats. *Eur J Pharmacol* 729: 17-21. [Crossref]
21. Ellman GL (1959) Tissue sulfhydryl groups. *Arch Biochem Biophys* 82: 70-77. [Crossref]
22. Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 47: 469-474. [Crossref]
23. Gulati K, Joshi JC, Ray A (2015) Recent advances in stress research: Focus on nitric oxide. *Eur J Pharmacol* 765: 406-414. [Crossref]
24. Hille R, Nishino T (1995) Flavoprotein structure and mechanism. 4. Xanthine oxidase and xanthine dehydrogenase. *FASEB J* 9: 995-1003. [Crossref]
25. Mohazzab H KM, Kaminski PM, Wolin MS (1997) Lactate and PO2 modulate superoxide anion production in bovine cardiac myocytes: potential role of NADH oxidase. *Circulation* 96: 614-620. [Crossref]
26. Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 87: 315-424. [Crossref]
27. Ray A, Chakraborti A, Gulati K (2007) Current trends in nitric oxide research. *Cell Mol Biol (Noisy-le-grand)* 53: 3-14. [Crossref]
28. Beckman JS, Koppenol WH (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad and ugly. *Am J Physiol* 271: C1424-C1437. [Crossref]
29. Schulz R, Dodge KL, Lopaschuk GD, Clanachan AS (1997) Peroxynitrite impairs cardiac contractile function by decreasing cardiac efficiency. *Am J Physiol* 272: H1212-H1219. [Crossref]
30. Yasmin W, Strynadka KD, Schulz R (1997) Generation of peroxynitrite contributes to ischemia-reperfusion injury in isolated rat hearts. *Cardiovasc Res* 33: 422-432. [Crossref]
31. Liu PT, Hock CE, Nagele R, Wong PYK (1997) Formation of nitric oxide, superoxide, and peroxynitrite in myocardial ischemia-reperfusion injury in rats. *Am J Physiol* 272: H2327-H2336. [Crossref]
32. Wang PH, Zweier JL (1996) Measurement of nitric oxide and peroxynitrite generation in the postischemic heart. Evidence for peroxynitrite-mediated reperfusion injury. *J Biol Chem* 271: 29223-29230. [Crossref]
33. Ferdinandy P, Panas D, Schulz R (1999) Peroxynitrite contributes to spontaneous loss of cardiac efficiency in isolated working rat hearts. *Am J Physiol* 276: H1861-H1867. [Crossref]
34. Pal R, Chaudhary MJ, Tiwari PC, Nath R, Babu S et al. (2016) Pharmacological studies on the anti-inflammatory and immunomodulatory role of pentoxifylline and its interaction with nitric oxide (NO) in experimental arthritis in rats. *Inflammopharmacology* 24: 221-231. [Crossref]
35. Pal R, Chaudhary MJ, Tiwari PC, Babu S, Pant KK (2015) Protective role of theophylline and their interaction with nitric oxide (NO) in adjuvant-induced rheumatoid arthritis in rats. *Int Immunopharmacol* 29: 854-862. [Crossref]