ROLE OF AMINOPHYLLINE ON DOXORUBICIN-INDUCED CARDIOTOXICITY IN ANIMAL MODEL

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ABSTRACT

Background: Doxorubicin is a potent chemotherapeutic drug. The clinical usefulness of doxorubicin has been limited largely by the risk of cardiomyopathy and life-threatening heart failure. Cellular changes leading to this toxicity are suggested to be mediated through a drug-induced increase oxidative stress.

Aim: This study investigated the effects of aminophylline on doxorubicin-induced cardiotoxicity in rats due to its antioxidant property.

Materials and methods: Thirty-five healthy Wistar strain albino rats were used. They were divided randomly into five groups (7 animals in each group). All animals supplied with standard food during the experiment with an access of water. They were distributed as follow: first group (normal saline treated group, 1 ml/kg, i.p.) in six equal doses in alternative days over a period of 2 weeks and considered as control group. Second, doxorubicin treated group (2.5 mg/kg, i.p., in six equal doses in alternative days over a period of 2 weeks to make a total cumulative dose of 15 mg/kg, body weight). The third, fourth and fifth groups were treated with aminophylline in doses (10, 20 and 30 mg/kg, i.p.) respectively plus doxorubicin (one hour prior each administered dose of doxorubicin). Blood samples were collected and used to determine the serum levels of cardiac biomarker cardiac specific troponin T in addition to oxidative stress parameters malondialdehyde (MDA) and superoxide dismutase (SOD).

Results: In aminophylline plus doxorubicin treated groups serum levels of cardiac troponin T and MDA were significantly lower than those of doxorubicin-treated group, While plasma SOD was increase. These changes occurred in a dose-dependent manner.

Conclusions: This suggests that aminophylline may have a role in the attenuation and prevention of the serious cardiac complications of doxorubicin.
Keywords: Aminophylline, Doxorubicin-induced cardiotoxicity, oxidative stress, antioxidant.

INTRODUCTION

Doxorubicin is a potent chemotherapeutic drug from the anthracycline antibiotics [1] that is used extensively for the treatment of haematological malignancies and solid tumours. [2] However, despite therapeutic efficacy, its clinical usage is limited by the development of cumulative dose-dependent cardiomyopathy [3] which may occur many years after the cessation of doxorubicin treatment [4] and that precludes some patients from receiving a highly effective treatment. Development of cardiotoxic side effects is associated with a poor prognosis. [5]

The exact pathogenesis of doxorubicin-induced cardiotoxicity is still not entirely clear although a diverse set of mechanisms have been proposed, including oxidative stress [6], intracellular calcium overload,[6] mitochondrial DNA damage, inhibition of protein synthesis, disturbance of myocardial adrenergic function, cytokine release, myofibrillar degeneration and cardiomyocyte apoptosis.[7] Among the multiple mechanisms, it is widely accepted that doxorubicin-induced cardiomyocyte apoptosis is primarily due to the generation of reactive oxygen species (ROS) in the myocardium which triggers intrinsic mitochondria-dependent apoptotic pathway in cardiomyocytes. [8,9]

The use of cardioprotective agents together with doxorubicin is a possible therapeutic approach. Several pharmacological agents have been shown to reduce the cardiotoxicity of doxorubicin, including antioxidants, iron-chelating agents and haematopoietic cytokines.[10-12] Very few, if any, agents have been proven to be applicable in clinical practice and the search for a cardioprotective drug to suppress doxorubicin-induced complications is going on. Aminophylline, a mixture of theophylline and ethylenediamine (85:15), is a bronchodilatating and antiasthmatic compound widely used in the treatment of bronchial asthma and chronic obstructive pulmonary disease. [13] Aminophylline is a non-specific phosphodiesterase inhibitor, thereby increasing tissue levels of cyclic AMP.[14] It has been indicated for the treatment of asthma and COPD.[15] Some previous studies reported that aminophylline exert some antioxidant activity in vitro [16] and others reported that aminophylline has the free radical scavenging effect in lung epithelial tissue. The present study investigated the possible protective effects of aminophylline in doxorubicin-induced cardiotoxicity in rat. Accordingly,
we carried out our present study on experimental rat models assuming that the results would have more or less similar implications in humans also.

MATERIAL AND METHODS

Study area

The present study was an animal model based case control study undertaken in the departments of Biochemistry with the collaboration of the department of Pharmacology of Burdwan Medical College, Burdwan, West Bengal, India.

Animal

Male Wistar strain albino rats (Rattus norvegicus albinus), between 1 to 2 months of age weighing 150 ± 12g, n = 35 were obtained from the appropriately maintained institutional animal house. The rats had free access to drinking water and rat food pellets. The light source in the animal room was regulated with 12 hr light period followed by 12 h dark schedule within a temperature of range of 22 to ± 2°C at a relative humidity of 45 to 50 %. All rats were acclimatized for at least 7 days before starting the present study. All procedures involving animals were performed in accordance with the ‘Guide for the Care and Use of Laboratory Animals (1985), NIH, Bethesda’ and ‘Guidelines for care and use of animals in scientific research’ by the Indian National Science Academy (INSA), New Delhi, India. The study was approved and permitted by the institutional ethics committee for care and use of laboratory animals, and started after obtaining the written consent from the concerned ethics committee [Memo No.BMC/2179/1 (6)].

Chemicals

Doxorubicin was from Sigma-Aldrich, St Louis, MO, USA and Aminophylline was from Turfarma (Turkey).

Study design

The animals had free access to food and drinking water till the day of start of the experiment. The rats were randomly divided, according to a table of random numbers, into five groups and proceed as shown in the Figure 1.
Figure 1: Study Design of the experiment.
Measurement of cardiotoxicity
The cardiac biomarker cardiac specific Troponin T (cTnT) is indicative for myocardiocyte damage and is currently used in the diagnosis and prognosis of myocardial ischemia.[17]
After 24hr from the last injection, about 3 ml of blood sample was obtained under light ether anaesthesia from each rat by cardiac puncture using disposable syringe. Each blood sample was placed in a gel tube and left to stand for 15-20 minutes at room temperature and used to obtain serum via centrifugation at 3000 rpm (round per minute) for 15 minutes and then preserved at -20 °C until determination the parameters of serum troponin T.

Measurement of oxidative stress
MDA, a marker of lipid peroxidation due to oxidative stress was measured by its reaction with thiobarbituric acid at 532 nm.[18] The serum levels of MDA were calculated using a calibration curve derived from 1,1,3,3- tetraethoxypropane (Fluka, Germany) as the external calibration standard. The calibration curve was linear in range from 1.25 to 2.5 nmol/ml (r2=0.997). Heparinised blood was used to obtained plasma and estimation of plasma superoxide dismutase (SOD) was done by the method of Kakkar et al. where one unit of SOD was defined as that amount of enzyme that inhibited the rate of electron transfer from NADH to nitroblue tetrazolium (NBT) by 50 % under specified conditions.[19]

Statistical analysis
The data for biochemical analysis was subjected to standard statistical analysis using the Statistical Package for Social Science (SPSS) 11.5 software for windows. For all tests, the p-value was considered to be significant if it was less than 0.05 at a confidence level of 95 %.

RESULTS
Effect of the administration of doxorubicin and different doses of aminophylline on cardiac troponin T levels.
As shown in figures 2 and Table 1, serum cardiac troponin T level was significantly (p< 0.001) higher in doxorubicin treated rats than the controls. The administration of aminophylline (10mg/kg, 20mg/kg and 30mg/kg i.p) one hour prior to each administered dose of doxorubicin showed a highly significant decrease (p< 0.001) in cardiac troponin T levels of Aminophylline treated rats as a dose dependent manner when compared with doxorubicin treated rats.
Effect of the administration of doxorubicin and different doses of aminophylline on serum MDA level.

As shown in figure 3 and Table 1, serum MDA level were significantly (p< 0.001) higher in doxorubicin treated rats than the controls. The administration of aminophylline (10mg/kg, 20mg/kg and 30mg/kg, i.p.) one hour prior to each administered dose of doxorubicin showed a highly significant decrease (p< 0.001) in serum MDA level of Aminophylline treated rats as a dose dependent manner when compared with doxorubicin treated rats.

Effect of the administration of doxorubicin and different doses of aminophylline on plasma SOD activity.

As shown in figure 4 and Table 1, plasma SOD activity were significantly (p< 0.001) higher in doxorubicin treated rats than the controls. The administration of aminophylline (10mg/kg, 20mg/kg and 30mg/kg, i.p.) one hour prior to each administered dose of doxorubicin showed
a highly significant decrease (p< 0.001) in plasma SOD activity of Aminophylline treated rats as a dose dependent manner when compared with doxorubicin treated rats.

![Graph showing plasma SOD activity](image)

Table 1: Serum troponin I levels of different treated groups.

<table>
<thead>
<tr>
<th>group</th>
<th>I (n = 7)</th>
<th>II (n = 7)</th>
<th>III (n = 7)</th>
<th>IV (n = 7)</th>
<th>V (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cTnT (µg/L)</td>
<td>0.09 ± 0.01*</td>
<td>1.7 ± 0.11</td>
<td>0.95 ± 0.12 *</td>
<td>0.29 ± 0.06*</td>
<td>0.38 ± 0.09*</td>
</tr>
<tr>
<td>Serum MDA (µmole/L)</td>
<td>2.76 ± 0.24*</td>
<td>12.49 ± 3.91</td>
<td>5.41 ± 0.96*</td>
<td>2.87 ± 0.39*</td>
<td>3.98 ± 0.47*</td>
</tr>
<tr>
<td>Plasma SOD activity (IU/ml)</td>
<td>3.18 ± 0.49*</td>
<td>6.69 ± 1.04</td>
<td>5.13 ± 0.52</td>
<td>4.63 ± 0.57*</td>
<td>3.72 ± 0.36*</td>
</tr>
</tbody>
</table>

Data was shown as mean ± S.D; p values were compared to Doxorubicin treated group; Asterisks indicate p<0.001.

**DISCUSSION**

The anthracycline antibiotic Doxorubicin is an important antineoplastic agent because of its high antitumor efficacy in hematological as well as in solid malignancies.[20] But its use is limited by the frequent induction of dose-dependent chronic cardiomyopathy. Oxidative stress has long been, and remains, the most studied and widely accepted cause of cardiotoxicity and some previous studies reported that aminophylline exert some antioxidant activity in vitro [16] so, this study was conducted to search the cardioprotective effects of aminophylline on doxorubicin-induced *in vivo* cardiotoxicity in rats.

In the present study, the cumulative dose of doxorubicin can induced cardiotoxicity as revealed from the increase in serum cardiac troponin T.
To find out the role of oxidative stress in doxorubicin induced cardiotoxicity, it was found that MDA levels and plasma SOD activity were increased. These results are consistent with studies in the animal studies.[21-23] Reactive oxygen species (ROS) were formed when the quinine moiety of doxorubicin is reduced to semiquinone, initiating a cascade of free radical formation that leads to many deleterious effects on cells, cell membranes and subcellular apparatuses.[24] Ultimately, these changes can lead to cell death and organ damage. The importance of cardiac mitochondria as key mediators of the cardiotoxicity of doxorubicin has been increasingly observed.[25] Doxorubicin impair mitochondrial calcium homeostasis, causing loss of stability of the mitochondrial membrane and ultimately, cell death.[26]

To search the cardioprotective effects of aminophylline it was revealed from the use of three aminophylline doses that there were decreased levels of the serum troponin I, MDA concentration and plasma SOD activity in a dose related manner. These results are in agreement with.[23,27,28]

The mechanisms by which aminophylline cause myocardial protection are not clear because of multiple pharmacological effects of this agents.[28] Aminophylline is a salt composed of two molecules of theophylline and one molecule of ethylenediamine. Therapeutic concentrations of aminophylline, are capable of antagonizing hypochlorous acid (HOCl) and moreover aminophylline at lower concentration was found to be effectively scavenging OH radical, because of its ethylenediamine component.[29] Theophylline has antioxidant effects also due to its non-selective phosphodiesterase inhibitory properties in human neutrophils.[30,31] The some non-oxidative mechanism of aminophylline to protect the Doxorubicin induced cardiotoxicity is to increase intracellular cAMP concentration by non-selectively inhibiting phosphodiesterase activity.[32] The increased intracellular cAMP phosphorylates sarcolemmal calcium channels.[33] Furthermore, Shahid and Rodger (1991), reported that cAMP was one of the essential factors of Ca2+ handling at reperfusion, and it inhibits Ca2+ overload.[34] Also Several lines of evidence indicate that an abnormal calcium handling of myocardial cells may explain, at least in part, the cardiac dysfunction seen in doxorubicin-induced cardiomyopathy [35].

CONCLUSION

The present study reported the cardioprotective effects of aminophylline against doxorubicin-induced cardiotoxicity in an in vivo rat model were reflected on the decreased levels of the serum cTnI and decrease blood MDA and SOD in a dose related manner that could explain
the widely accepted theory that doxorubicin-induced cardiomyocyte apoptosis is primarily due to generation of oxidative stress. This suggests that aminophylline may have a role in the attenuation and prevention of the serious cardiac complications of doxorubicin. The combination of aminophylline with doxorubicin is a novel strategy that has the potential for protecting against doxorubicin-induced cardiotoxicity in clinical practice.

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REFERENCES
We do not have any conflict of interest.


