Research Article

Effect of xanthine oxidase inhibitors on cardiac toxicity in cyclophosphamide-treated rats

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Abstract
There is no clear data addressing the effect of xanthine oxidase (XO) inhibitors on cyclophosphamide (CYC)-induced cardiotoxicity. This study was aimed to study the effect of allopurinol (ALL) on CYC-induced cardiotoxicity in rats. Rats were allocated into 4 groups: group 1: control, group 2: ALL group (recieved ALL 100 mg/kg/day orally for 14 days), groups 3: CYC group (received CYC 200mg/kg ip single dose at 9th day) and group 4: ALL+CYC group: received both ALL plus CYC in the same previous doses. Rats were sacrificed after 2 weeks. The cardiotoxic effect of CYC was assessed by serum level of lactic dehydrogenase (LDH). The effect of CYC on the pathogenesis of cardiotoxicity was evaluated by measuring cardiac malonaldehyde (MDA) and catalase levels. Cardiotoxicity induced by CYC revealed statistically significant improvement in rats treated with ALL compared with CYC alone group. These results suggest that xanthine oxidase inhibitors as ALL can protect against CYC- induced cardiotoxicity. Their effects rely, at least partially, on their anti-oxidant effects.
Key Words: xanthine oxidase, cardiotoxicity**,** cyclophosphamide

Introduction
Cancer is a dangerous and fatal disease that is more common in old age (Berger et al., 2006). The second leading cause of long-term morbidity and death among cancer survivors is cardiovascular complications caused by chemotherapeutic agents (Jemal et al., 2010; Seigel et al., 2012; Bodai and Tuso, 2015). CYC is a common chemotherapeutic agent used in the treatment of nonmalignant, as well as malignant conditions including lymphoma, breast cancer, ovarian carcinoma, leukemia, multiple myeloma and mycosis fungoides (Emadi et al., 2009).

Cardiotoxicity is a common adverse effect that occurs with CYC as a result of many mechanisms, including oxidative stress process with production of free radicles (Dhesi et al., 2013). XO is implicated in reactive oxygen species generation and inhibition of XO has been proposed as a mechanism for improving cardiovascular health (Dawson and Walters, 2006). XO inhibitors, as ALL (Pacher et al., 2006) act by decreasing the production of uric acid, which has harmful effects on cardiovascular system. In addition they inhibit oxidative stress process and production of free radicals (Nakatsu et al., 2015), thus, they might play a role in decreasing toxic effects of CYC on the heart.

Materials and Methods
Drugs, chemicals, and kits
Allopurinol 10g powder (a kind gift from EPICO Co., Egypt), Carboxy methylcellulose (El-Nasr Pharmaceutical Co., Egypt), Catalase kit (Biodiagnostic, Egypt), CYC vial 1g (Baxter oncology GmbH, Germany) and LDH kit (Spectrum diagnostic, Egypt).

Animals
The present study was conducted on adult male albino rats weighing 165–225 g. They were obtained from the National Research Centre, Giza, Egypt. They were housed in laboratory cages with free access to water. They were fed a standard diet of comercial rat chow and left to accommodate to the environment for one week before the start of the experiments.

Experimental protocol

The rats were weighed and assigned into four groups (n=7-9), each receiving the following treatments: **Group 1: Control group** (non-treated) received carboxy methylcellulose (vehicle of ALL) orally and saline (vehicle of CYC) ip, **Group 2:** ALL group received both ALL (100 mg/kg/day orally) for 2 weeks that is suspended in carboxy methyl cellulose and the vehicle of CYC, **Group 3:** CYC group received a single dose of CYC (200 mg/kg ip.) dissolved in saline at the ninth day and served as intoxicated positive control group, **Group 4:** CYC/ALL group received combination of ALL (100 mg/kg/day orally) for 2 weeks and CYC (200 mg/kg ip.) at the ninth day of the experiment.

The above mentioned doses of CYC and ALL were selected on the basis of our preliminary studies, as well as previously published results (Mansour et al., 2015; Sagor et al., 2015), respectively.

Samples collection and preparation

At the end of the experimental period, the animals were scarified. Blood samples and heart specimens were collected.

- Sera were separated by centrifugation (centrifuge Jantezki, T30, Germany) for 10 min at 5000 rpm and used for determination of lactic dehydrogenase (LDH)
- Heart tissues were homogenized in approximately 2ml. of ice-cold saline and 10 volumes of ice-cold phosphate buffer (prepared by dissolving 8.01g NaCl, 0.20g KCl, 1.78g Na_2HPO_4. $2H_2O$ and 0.27g KH_2PO_4 in 1 liter of distilled water and pH was adjusted at 7.4), respectively, using a polytron homogenizer (Tri-R Stir-R homogenizer, Tri-R Instruments, Inc., Rockville Centre, NY). Aliquots were prepared and stored at -80^{o}C until estimation of malondialdehyde (MDA) and catalase.

Assessment of the biochemical parameters

Determination of cardiac enzymes in the form of serum LDH: The level of LDH was determined using LDH kinetic kit according to the basic principles described by Buhl and Jackson (1978).

Determination of cardiac oxidative stress parameters

Determination of lipid peroxides in the form of malonaldehyde (MDA) in heart:

The MDA is a reactive aldehyde that is a measure of lipid peroxidation. Cardiac contents of MDA were determined using the thiobarbituric acid method described by Mihara and Uchiyama method (1978).

Assessment of catalase in heart:

Catalase activity was measured using colorimetric catalase kit according to Aebi, 1984.

Statistical Analysis of the data

Results were expressed as means ± SEM. Results were analyzed by one-way ANOVA followed by Tukey's test. Differences with p value < 0.05 were considered significant. Graph Pad Prism was used for statistical analysis (version 5.01 for Windows, Graphpad Software, San Diego California USA;www.graphpad. com)

Results

Assessment of the biochemical parameters

Assessment of serum cardiac enzymes in the form of LDH:

The results of the current study revealed that administration of ALL alone did not affect cardiac serum enzyme; LDH, compared to control. On the other hand, administration of CYC (200mg/kg) caused a significant increase in serum levels of LDH as compared with control group. Co-administration of either ALL (100mg/kg) with CYC significantly decreased LDH level as compared with CYC-intoxicated group **(Table 1)**.

Determination of oxidative stress parameters

Effect of cyclophosphamide, allopurinol and their combinations on MDA level in rats:

In the current study, heart MDA was not affected by sole treatment with ALL. To the contrary, cardiac MDA was significantly increased in CYC-intoxicated group compared to control. Treatment with ALL significantly attenuated the effect of CYC on cardiac MDA in comparison to CYC-intoxicated group **(Table 2)**.

Effect of cyclophosphamide, allopurinol and their combinations on catalase level in the heart in different rat groups:

In the current study, single treatment with ALL or CYC, as well as combined treatment with CYC/ALL did not significantly affect cardiac catalase level compared to control **(Table 2)**.

Table (1): Effect of cyclophosphamide (CYC), allopurinol (ALL) and their combinations on serum lactic dehydrogenase (LDH) level

Groups	LDH (U/L)
Control	201±108
ALL	341±41
CYC	1234±617a
CYC/ALL	200±41b

Results represent the mean ± S.E.M (n= 5-9). [a] Significant difference from control group, [b] significant difference from CYC group ($P < 0.05$).

Table (2): Effect of cyclophosphamide (CYC), allopurinol (ALL) and their combinations on malondialdehyde (MDA) and catalase levels in heart in rat.

Groups	Cardiac MDA (nmol/g tissue)	Cardiac catalase (U/g tissue)
Control	**163±10**	**61±2**
ALL	**255±26**	**53±7**
CYC	**609±31[a]**	**52±7**
CYC/ALL	**292±26[a,b]**	**49±6**

Results represent the mean ± S.E.M (n= 5-9). [a] Significant difference from control group, [b] significant difference from CYC group ($P < 0.05$).

Discussion

Cardiotoxicity is the occurrence of heart electrophysiological dysfunction or myocardial damage. It may be caused by chemotherapy treatment (López-Fernández and Thavendiranathan, 2017). CYC is an effective and widely used chemotherapeutic agent used in the treatment of malignant diseases and autoimmune disorders (Xu and Xuewu, 2015). However, its clinical use is limited because of its serious multiple organ toxicities including cardiotoxicity (Bhatt et al., 2017).

The current study evaluated the effects of a purine XO-inhibitor, ALL, on CYC-induced cardiac toxicity in rats. The design of this study offers the advantage that it allowed us to record the early evidences of cardiac damage in the form of elevation of cardiac enzymes, in addition to record the late evidence of cardiac toxicity in the form oxidative stress processes.

Our data showed that CYC-induced cardiotoxicity was manifested by significant elevation in the level of LDH. Viswantha Swamy and his associates (2013) reported that CYC increased serum LDH through its cardiotoxic effect, by inducing a direct myocardial endothelial damage and destruction of myocardial cells. As a result this enzyme is released into the blood stream and serves as the diagnostic marker of myocardial tissue injury.

Interestingly, our results showed that co-administration of ALL with CYC-intoxicated rats improved the cardiotoxicity, as evident by significant reduction in serum level of LDH as compared to CYC-intoxicated group.

Oxidative stress is an indicator of the damage that results from a change in the balance between oxidants and antioxidants in favor of oxidants. If the delicate balance

between oxidants and anti-oxidants cannot be maintained in tissues, many pathological changes extending to cellular damage occur (Mukherjee et al., 2013). Reactive oxygen species are generated by various biological systems, including xanthine oxido-reductase and contribute to many physio-logical and pathological phenomena. Mammalian xanthine dehydrogenase (XDH) can be converted to XO, which produces both superoxide anion and hydrogen peroxide (Nishino et al., 2008).

Oxidative stress, caused by ROS, is linked to the direct effects of CYC and CYC-induced toxicity that increases lipid peroxidation in different tissues, including heart in rats (Mansour et al., 2015). The ROS, thus formed, may lead to cellular damage by peroxidation of membrane lipids, sulfhydryl enzyme inactivation, protein cross-linking and DNA breakdown (Mansour et al., 2015).

In the current study, CYC also significantly increased cardiac tissue MDA level, which is in line with previous studies (Avci et al., 2017). Here, pretreatment with ALL improved cardiac MDA levels. Our study was supported by Wang and his colleagues (2016) who reported that ALL improves cardiac function in the setting of congestive heart failure following myocardial infarction and the inhibition of XO by ALL attenuated the injury caused by ROS and decreased MDA level in the heart of rats.

In our study, CYC did not affect cardiac catalase, to the contrary of previous results (Viswanatha Swamy et al., 2013; Ogunsanwo et al., 2017). It is possible that the discrepancy in our results is due to different dose administration procedures, rat strain or environmental factors. Here, ALL also did not significantly affect catalase level.

Taken together, the present study concluded that ROS and XO enzymatic pathways may largely participate in the mechanism of pathogenesis of cardiac toxicity related to CYC administration. In addition, XO-inhibitors as ALL can ameliorate CYC-induced cardiotoxicity.

Conclusion

From the above data, it is clear that XO activity may be one of the mechanisms by which CYC may cause cardiac alterations. ALL was able to attenuate the cardio-toxicity induced by CYC, at least in part through XO inhibition and anti-oxidant mechanisms.

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