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RESEARCH ARTICLE

THE POSSIBLE PROTECTIVE EFFECT OF PENTOXIFYLLINE AGAINST DOXORUBICIN-INDUCED HEPATOTOXICITY IN RABBITS

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Abstract

Background **:**the chemotherapeutic activity of doxorubicin in treatment of different types of cancer has a clinical limits due to, in part, kidney and liver toxicity in addition to cardiotoxicity so many strategies are tried to reduce this toxicity and use of pentoxifylline may be one of these.

Objective: this study was designed to assess the possible protective effect of pentoxifylline against renal oxidative stress and dysfunctions induced by doxorubicin administering in rabbits model.

Methods: measuring the oxidative stress parameters that included malondialdehyde and reduced glutathione in the liver tissue homogenate, in addition to liver function serum transaminase enzymes.

Results: analysis of data by using independent t-test showed a significant improvement of oxidative stress and biochemical parameters evidenced by a significant reduction of malondialdehyde and a significant elevation of glutathione by administering of pentoxifylline prior and after doxorubicin administration and a significant reduction of transaminase enzymes (aspartate aminotransferase and alanine aminotransferase) as compared to doxorubicin group.

Conclusion: the presented data indicated that pentoxifylline has a protective effect in doxorubicin-induced liver toxicity in rabbits.

Introduction

The introduction of anthracyclines to the chemotherapy of malignant neoplasms has been one of the major successes of cancer medicine but still with severe toxic side effect [1] .The mechanism of toxicity included the oxidative stress status that is characterized by excessive production of reactive oxygen species (ROS) and/or a reduction in antioxidant defenses causing an imbalance in the normal metabolism of oxygen. The addition of one electron to the quinone moiety of doxorubicin (DOX) leads in the formation of a semiquinone form that quickly regenerates the quinone form, by reducing molecular oxygen to ROS. These ROS can generate both hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$), which attack DNA and oxidize DNA and induce apoptosis in cancerous and normal tissue cells [2]. Anthor pathway of DOX toxicity through interfering with non-enzymatic

metabolic reactions in which iron is involved, can alter the metabolism of iron. Subsequently, increment of free iron level can react with DOX and formation of DOX-iron complex and leads to ROS production [3]. This complex is highly toxic to membrane lipids, proteins and DNA [4]. The increased ROS leads to mitochondrial calcium overloading, promotes mitochondrial pore opening, causes mitochondrial swelling and ATP depletion, and triggering necrotic cell death [5].

Pentoxifylline (PTX), a methyl xanthine derivative, for the treatment of intermittent claudication on the basis of chronic occlusive arterial disease of the limbs [6]. PTX is an inhibitor of phosphodiesterase (PDE). PTX has been reported to suppress the production of tumor necrosis factor-alpha (TNF-α) and modulate the production of other inflammatory cytokines [7]. PTX might prevent liver toxicity and the oxidative damage by reducing oxidative stress and increasing antioxidant enzyme levels [8] [9] [10].

MATERIALS AND METHODS

Animal used

Eighteen domestic rabbits of either sex weighing between (1 - 1.5) kg were used. Animals were housed under standard conditions of temperature and relative humidity with a 12:12 light: dark cycle All procedures involving animals were carried out under the institute ethics committee approval.

Experimental design

Animals were divided into three groups, each of six animals as follow: **Group1:** control group which recieved (5) ml of isotonic normal saline, then animals were sacrificed at day 5 .**Group2:** DOX (Ebewe Pharma, Austria(50mg/25ml)) group which treated with single dose of DOX (25mg/kg/i.p.) at third day, then animals were scarificed (after 48hour) at day 5.**Group 3:** PTX (Sanofi- Aventis, Turkey(100mg/5ml)) treated rabbits with (80mg /kg/ i.p. /daily)[8,11] three successive days prior and two three successive after DOX (25mg/kg/i.p.) that was given at day 3, then animals were scarificed at day 5.

Preparation of serum samples

The blood was collected by cardiac puncture at day 5, then centrifuged for 15-20 minutes at 3000 rpm and the supernatant was used for the estimation of liver transaminase enzymes. After the animals were euthanized by anesthetic ether, livers were quickly excised, placed in chilled phosphate buffer solution (PH 7.4) at 4 ^{0}C, blotted with filter paper and weighed.

Preparation of tissue homogenate

One gram of organ was took to prepare 10% tissue homogenate using the phosphate buffer solution (PH 7.4) by utilizing tissue homogenizer [12] at set 3 for 1 minute at 4 ^{0}C. All preparations were freshly prepared and kept frozen (-70 ^{0}C) unless worked immediately.

Measurement of lipid peroxidation

Malondialdehyde (MDA), the end product of lipid peroxidation, was analyzed according to the method of Buege, and Aust [13]. Light absorbency of clear-supernatant solution was determined at 535 nm against a blank using spectrophotometer and the results were expressed as (nmol/g tissue).

Determination of reduced glutathione Level

Total thiol groups contents, which could be used as indicator for reduced glutathione (GSH), was determined according to the method of Elman's [14]. The light absorbency of the solution at 412 nm was measured after 2 minutes.

Determination of serum AST and ALT

Serum transamines enzymes(AST and ALT) was determined according to the method of Reitman and Frankle in 1975 [15] , using ready-made kit for this purpose.

Statistical analysis

Mean values ± S. E. M. were calculated for each parameter. For the determination of significant differences, each parameter was analyzed separately and independent Student's t -test for comparison between two groups was carried out by SPSS version 20. P value < 0.05 was considered significant.

RESULTS

Rabbit's treated with single dose of DOX (25 mg/kg), showed a significant elevation of the MDA contents of liver tissue homogenate (304.5%)and a significant reduction of GSH contents of liver tissue homogenate (276.2 %) compared to the control group (P<0.05), [table 1 , figure (1 and 2)] . DOX treated group showed a significant elevation of the levels of serum AST and ALT being(233.7 % and 98.1 % respectively) compared to the control group (P<0.05) , [(table 1), figures (3) and (4)] . Rabbits were treated with once daily dose of PTX (80mg/kg/i.p.) for three successive days before and two successive days after DOX administration showed a significant reduction of the MDA contents (154.%)of liver tissue homogenate and a significant elevation of GSH(33.38) contents of liver tissue homogenate compared to the DOX group (P<0.05), [table (1) , figure (1 and 2)] . It also showed a significant reduction of the serum levels of AST (57.58%)and ALT (42.5%)being compared to that observed in DOX treated group ,but it still significantly different in level of MDA,GSH ,AST and ALT (154.3 % ,-33.38 %, 75.58 % and 42.5% respectively) compared to control group (p<0.05), [(Table 1), figures (3 and 4)].

Table (1) the effect of pentoxifylline on the levels of MDA, GSH, AST and ALT levels of the study groups.

Groups N=6/each group	MDA level (µmol/g) and percent of change	GSH level (µmol/g) and percent of change	AST level (U/L) and percent of change	ALT level (U/L) and percent of change
Control	23.87 ± 1.5	14.84 ± 0.54	25.8 ± 1.62	54.8 ± 4.81
DOX-treated(25 mg/kg)	60.02 ± 2.69*	6.5± 0.56*	86.1 ± 3.29 *	108.6 ± 2.06*
PTX-treated group (80mg/kg/i.p.)	37.74 ± 1.3*s	15.9 ± 1.35*^{s}	45.3 ± 2.02*^{s}	31.5 ± 0.92 *^{s}

[Each value represents mean ± SEM. N = number of animals in each group. (*): significant difference at $p \leq 0.05$ with respect to the control group. (^{S}): significant difference at $p \leq 0.05$ with respect to the DOX treated group. Percent of change = (treated - control /control) x 100)[16]].

DISCUSSION

It has been investigated, that the use of DOX results in an increased production of free radicals, which have a great potential to react rapidly with lipids that cause lipid peroxidation [17]. Free radicals and reactive oxygen's species can damage hepatocyte membranes and which can elevate intracellular Ca^{+2} and liver cell death [18]. Enhanced lipid peroxidation is known to be one of the toxic manifestations of DOX administration and is measured in terms of MDA levels a lipid peroxidation marker .Excessive lipid peroxidation has been reported in DOX-treated rabbits and showed significantly increased levels of MDA in both kidney and liver tissues compared to control group.

GSH is a tripeptide, an antioxidant and plays an efficient role in the detoxification of ROS, conjugation and excretion of toxic compounds. Reduction of GSH level in tissues may result in peroxidative injury and damage of the cellular defense against ROS. In the present study findings are consistent with previous reports that showed GSH concentration is decreased upon DOX treatment [19-22] .The liver toxicity of DOX was estimated by elevation of serum transaminases which well known as a sensitive markers of liver damage. Membrane permeability and transport functions are altered by injured hepatocytes, which lead to the leakage of enzymes from the cells that caused an increment in the levels of ALT and AST in serum. Administration of DOX to rabbits significantly

increased serum ALT and AST levels when compared with the control group [23, 24]. The increased activity of serum AST is a well-known diagnostic marker of myocardial function due to DOX cardiotoxicity [25].

Karimi et al (2014), showed the intracellular cAMP-elevating agents like PTX, may be considered beneficial for the protection or recovery against agent induced toxic damage in liver mitochondria [9]. Ranjbar et al (2013) ,demonstrated that the activity of catalase and superoxide dismutase enzyme decreased by PTX-antioxidant effect which protected the liver mitochondria from toxic effects [10] .

In this study demonstrated the protective effect of PTX, by inhibiting the oxidative stress due to its antioxidant effects on lipid peroxidation as evidence by decrease in MDA content and the ability for preserving GSH levels in liver tissue homogenate, this results are compatible with previous studies [8, 11] .Elevations of the hepatic enzymes were dose dependently reduced by PTX with near normalization of transaminase enzymes levels. The useful therapeutic effect of PTX, holds a consistency with the other mentioned studies [26, 27]. It was observed that PTX showed a significantly declined the extent and severity of liver injury. This effects may be attributed to a reduction in oxidative stress markers, increased blood flow and irrigation to the liver and increase chance for liver vitality and finally, reduction of the expression of TNF-α, TNF-α type-1 receptors, and NF-κB [26]. Thus TNF-α inhibition may be one of the therapeutic interventions to overcome the deleterious effects of on liver. Hepatocellular injuries caused by TNF-α are not fully defined ,which may mediate direct mitochondria toxicity, and induce apoptotic or necrotic cell death .Generation of ROS formed mainly by the mitochondria is another factor that could contribute to the cytotoxic effects of TNF-α [26,28,29] . PTX has been reported to suppress the production of tumor necrosis factor-alpha (TNF-α) and modulate the production of other inflammatory cytokines [7].

CONCLUSION

Pentoxifylline has a protective and therapeutic effect against the liver toxicity caused by doxorubicin.

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