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Attenuation of the Acute Adriamycin-induced Cardiac and Hepatic Oxidative Toxicity by N-(2-Mercaptopropionyl) Glycine in Rats

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The protective effect of the synthetic aminothiol, N-(2-mercaptopropionyl) glycine (MPG) on adriamycin (ADR) induced acute cardiac and hepatic oxidative toxicity was evaluated in rats. ADR toxicity, induced by a single intraperitoneal injection (15 mg/kg), was indicated by an elevation in the level of serum glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), creatine kinase isoenzyme (CK-MB), and lactic dehydrogenase (LDH). ADR produced significant elevation in thiobarbituric acid reactive substances (TBARS), indicating lipid peroxidation, and significantly inhibited the activity of superoxide dismutase (SOD) in heart and liver tissues. In contrast, a single injection of ADR did not affect the cardiac or hepatic glutathione (GSH) content and cardiac catalase (CAT) activity but elevated hepatic CAT. Pretreatment with MPG, (2.5 mg/kg) intragastrically, significantly reduced TBARS concentration in both heart and liver and ameliorated the inhibition of cardiac and hepatic SOD activity. In addition, MPG significantly decreased the serum level of GOT, GPT, CK-MB, and LDH of ADR treated rats. These results suggest that MPG exhibited antioxidative potentials that may protect heart and liver against ADR-induced acute oxidative toxicity. This protective effect might be mediated, at least in part, by the high redox potential of sulfhydryl groups that limit the activity of free radicals generated by ADR.

Keywords: Adriamycin; N-2-mercaptopropionyl glycine; Lipid peroxidation; Antioxidants; Creatine kinase; Oxidative stress; Heart; Liver

INTRODUCTION

Adriamycin (ADR), an anthracycline glycoside antibiotic, belonges to doxorubicin group. It is a potent chemotherapeutic agent used for the treatment of a wide spectrum of human cancers.[1] However, cardiotoxicity is one of the the most serious adverse effect limiting the use of all anthracyclines.[2] Recent studies suggest that

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ADR cardiotoxicity results from a combination of profound oxidative stress due to reactive oxygen species (ROS) and unusual sensitivity of mammalian heart to cytotoxic effects of the oxidative stress. ADR is a quinone and can be converted to a semiquinone by mitochondrial, lysosomal and cytosolic enzymes.[3] Semiquinone is a charged moiety that readily donates an electron to an oxygen molecule, resulting in the generation of superoxide anion ($O_2^{-\cdot}$) which is reactive and dismutes to toxic hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). H_2O_2 can be converted further to highly toxic hydroxyl radicals ($^{\cdot}OH^-$) in the presence of transition metal, most often Fe^{2+}.[4] These ROS are toxic and react with lipids, proteins and nucleic acids, resulting in lipid peroxidation (LPO), depletion of sulfhydryl-containing peptides, and damage to DNA. Mammalian myocardium is somewhat deficient in catalase (CAT), glutathione (GSH) peroxidase and GSH transferase, compared to the kidney and liver. There is some evidence that ADR metabolites have damaging effects on these antioxidant enzymes.[2]

One of the approaches used for minimizing ADR cytotoxicity is coadministration of protective antioxidant agents. An extensive array of antioxidants has been evaluated for their potential to reduce ADR toxicity on the basis of a perceived ability to modulate the biochemical alterations that accompany ADR induced oxidative stress. In a preliminary animal study, amifostine, an aminothiol that scavenges superoxide anions and hydroxyl radicals, has been found to offer cardioprotection against doxorubicin induced cardiomyopathy.[5]

N-(2-Mercaptopropionyl) glycine (MPG), a detoxicating synthetic aminothiol and antioxidant with a wide range of clinical applications, is an effective radioprotector against radiation-induced oxidative damage.[6,7] It is known as tiopronin and has been used in experimental cardioprotection[8–10] *in vivo* and *in vitro* because it is effective at very low doses (20 mg/kg), far below the toxic dose of 2100 mg/kg. Therefore, the aim of the present study was focused on the ability of the sulfhydryl compound MPG, as oxyradical scavenger, to reduce ADR induced toxicity and oxidative stress in two organs: heart, as the most sensitive site to ADR action, and liver, as the most important free radical scavenger organ.

MATERIALS AND METHODS

All the chemicals were of reagent grade and were obtained from Sigma Chemical Co., USA. Four months old adult male rats, weighing 120–150 g, bred at our experimental animal care center, were included in the study. They were maintained under standard condition and fed a standard chow and water *ad libitum*. All experiments were carried out in accordance with protocols approved by the local experimental animal ethics committee. Rats were randomly divided into six groups of eight animals each, as follows.

Group 1: received 0.5 ml water intragastrically (ig) daily for seven days and served as control group. Group 2: received MPG (2.5 mg/kg body weight, ig) dissolved in distilled water daily for seven days.[9] Group 3: received single intraperitoneal (ip) injection of ADR (15 mg/kg).[11] Group 4: pretreated with MPG for seven days followed by ADR treatment. ADR was injected to rats in the 3rd group 30 h before decapitation. To group 4, ADR was injected simultaneously with the last dose of MPG. In each case animals were sacrificed by decapitation 30 h after the last treatment.

Blood was collected from carotid arteries and the sera were separated for estimation of serum activities of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT),[12] creatine kinase isoenzyme (CK-MB),[13] and lactic dehydrogenase (LDH).[14] The heart and liver were isolated, weighed, cooled and homogenized for biochemical determinations of the product of lipid peroxidation thiobarbituric acid reactive substances (TBARS),[15] the activity of SOD,[16] CAT activity[17] and GSH content.[18]

Statistical Analysis

Statistical comparison was made using analysis of variance (ANOVA) followed by Newman–Keuels post hoc test.

RESULTS

The results of the present study as shown in Tables I–III revealed that the administration of MPG (2.5 mg/kg, ig) daily for seven days induced insignificant changes in the tested parameters as compared with the control except the hepatic SOD which displayed a significant elevation.

Table I shows the effect of MPG (2.5 mg/kg, ig) on ADR (15 mg/kg, ip) induced biochemical changes in the serum of the treated rats. Animals that received single injection of ADR had significantly higher levels of GOT, GPT, CK-MB, and LDH when compared with the control values. Administration of MPG daily for seven days before ADR treatment prevented the changes recorded in the activities of these enzymes in the serum and displayed insignificant changes when compared with the control.

In addition, a significant increase in LPO was induced in heart and liver, as indicated by an increase in TBARS concentration in both tissues, after 30 h of ADR treatment (Tables II and III). The present results also revealed a good amelioration of LPO in both tissues against ADR induced rise in TBARS level by oral administration of MPG daily for seven days before ADR treatment.

The activity of SOD in heart and liver was significantly inhibited after 30 h of ADR treatment (Tables II and III). This effect was significantly prevented in both tissues by the administration of MPG before ADR treatment and was significantly elevated in liver only as compared with the control group. The activity of CAT in heart did not change after 30 h of ADR treatment (Table II). On the other hand, CAT activity in liver was significantly increased in the liver of the same ADR-treated rats. This effect was significantly modulated in liver by oral administration of MPG before ADR treatment (Table III). The non-enzymatic antioxidant GSH did not change in heart or liver in the same ADR-treated rats 30 h after treatment (Tables II and III).

DISCUSSION

The present study demonstrates that MPG administered in combination with ADR is an effective scavenger of toxic free radicals (FR), inhibitor of LPO and might serve as a novel antioxidant agent against ADR-induced oxidative tissue injury.

LPO is induced in animal tissues after the administration of ADR.[19] Furthermore, Singal *et al.*[3,20–22] have shown that FR scavengers are useful in protecting against ADR toxicity. These studies indicate that oxidative stress is increased after ADR treatment due to over-

TABLE I Effect of MPG (2.5 mg/kg) on ADR (15 mg/kg)-induced biochemical changes in serum of rats. (Values are mean ±SEM ($n = 8$))

Treatment	Serum enzyme levels (U/L)			
	GOT	GPT	CPK-MB	LDH
Control	79.0±3.5	14.6±1.9	10.5±1.3	260±19.3
MPG	75.5±4.5	13.2±1.4	10.7±1.02	271±21.3
ADR	105.4±7.4*	37.2±2.3*	36.6±1.8*	377±30.1*
MPG + ADR	77.5±6.9†	14.2±0.9†	18.8±1.5*†	271±20.0†

*Significant as compared with control group, $P < 0.01$.
†Significant as compared with ADR-treated group, $P < 0.01$.

TABLE II Effect of MPG (2.5 mg/kg) and ADR (15 mg/kg) on the LPO (nmol TBARS/g fresh tissue), SOD, (U/g fresh tissue), CAT (U/g fresh tissue) and GSH (mg/g fresh tissue) in rat heart. (Values are mean ±SEM of eight animals.)

Treatment	Parameter levels			
	TBARS	SOD	CAT	GSH
Control	94±9	1328±98	0.109±0.03	0.510±0.06
MPG	82.6±7	1355±42	0.103±0.03	0.490±0.05
ADR	355±40*	625±40*	0.115±0.03	0.512±0.04
MPG + ADR	156.8±19*,†	915± 57*,†	0.108±0.03	0.591±0.05

*Significant as compared with control group, $P < 0.05$.
†Significant as compared with ADR-treated group, $P < 0.05$.

production of ROS and decreased efficiency of endogenous antioxidant defenses in heart, liver, kidney and brain.[23] ADR has been demonstrated to be a potent generator of FR by either enzymatic pathway or by forming an ADR–Fe^{+3} complex.[2,24] Results of the present study show that there was an increase in LPO indicated by increased TBARS concentration and lower SOD activity in heart and liver after 30 h of ADR administration. Our results are consistent with those from other studies[22,25] which reported increased LPO following ADR treatment in heart and liver[23,26] and in nervous system.[27] Moreover, Hershko *et al.*[28] have reported enhanced iron-dependent LPO with ADR in heart. The ADR–iron complex is highly toxic to various intracellular proteins and membrane lipids.

In addition, extracellularly generated FR may initiate toxic reactions with unsaturated fatty acids present in bilayer core and with membrane proteins containing oxidizable amino acids leading to changes in hydrophobic interactions between adjacent proteins and phospholipids. It has been also suggested that increased membrane permeability caused by LPO or oxidation of important membrane proteins can cause a breakdown of transmembrane ion gradients and inhibition of integrated cellular metabolic processes. Moreover, it has been reported that FR enhance calcium release from the sarcoplasmic reticulum and also that they inhibit sarcolemmal Na^+ K^+ ATPase possibly causing the activation of Na^+ Ca^{2+} exchange mechanism in the myocardium.[29,30] Finally significant degradation of membrane function and structures may favor apoptosis.

The activities of serum CK-MB and LDH have been widely used as parameters for the diagnosis of cardiac dysfunction. A single dose of ADR (15 mg/kg, ip) produced significant elevation of serum GOT, GPT, CK-MB and LDH levels at 30 h after ADR treatment. It is reported that serum activities of these enzymes are increased in several types of heart injury such as myocardial infarction or myocarditis and heart failure.[13]

TABLE III Effect of MPG (2.5 mg/kg) and ADR (15 mg/kg) on the LPO (nmol TBARS/g fresh tissue), SOD, (U/g fresh tissue), CAT (U/g fresh tissue) and GSH (mg/ g fresh tissue) in rat liver. (Values are mean ±SEM of eight animals.)

Treatment	Parameter levels			
	TBARS	SOD	CAT	GSH
Control	85±7.3	1128±74	0.100±0.01	3.58±0.25
MPG	82±6.0	1397±90*	0.110±0.01	3.38±0.28
ADR	390±35.5*	990±60*	0.138±0.03*	3.08±0.35
MPG + ADR	207±16.6*,†	1300±70*,†	0.095±0.02†	3.10±0.20

*Significant as compared with control group, $P < 0.05$.
†Significant as compared with ADR-treated group, $P < 0.05$.

This response might be attributed to notation that ADR-induced FR may attack the cardiac membrane and cause protein and/or LPO, which would compromise the cellular integrity and potentially account for the increase in serum CK-MB and LDH activities. This effect might contribute, together with ADR-induced oxidation of CK isoenzymes in heart, to the decrease of CK activity in cardiomyocytes.[31] It has been suggested that cardiomyocytes and hepatocytes utilize the creatine phosphate energy shuttle to sustain a constant level of ATP that is appropriate to maintain cardiac and hepatic performance. The increase in serum CK-MB and LDH indicating the leakage of these enzymes through the membranes by ADR coincide with previous study. It is demonstrated that the increase of CK level in serum and in myocytes culture media as a result of possible cell damage is occurring in concert with the decrease of CK activity in the cardiac tissue.[31–33] Thus, alteration of myocyte and hepatocyte energetics might be implicated in ADR oxidative toxicity.

In the present study, the increase in LPO was accompanied by a concomitant decrease in the activity of SOD in heart and liver 30 h after ADR treatment. These results are in agreement with those of Yen *et al.*[32] who reported a decline in the activity of Mn-SOD in ADR-treated rats and concluded that mitochondria is the critical site of cardiac injury mediated by ADR-induced inhibition of Mn-SOD. However, up-regulation of antioxidant gene expression occurred in response to ADR in mouse heart was observed, although the antioxidant activities were not all increased.[34] In this study, the CAT activity was unaffected in rat heart but was significantly elevated in liver 30 h after ADR treatment (Tables II and III). Yin *et al.*[34] and Venkatesan[35] demonstrated increased CAT activity in heart after four and two days respectively. Thus, the effect of ADR on the CAT might be time dependent. In addition, the difference of the effect of ADR on CAT activity in heart and liver after 30 h of ADR administration might explain the higher resistance of liver as compared to heart with respect to ADR-induced toxicity recorded in the present study.

Furthermore, the present results demonstrated an insignificant change in GSH content in heart and liver at 30 h after ADR treatment with high LPO and decreased activity of SOD in both organs. However, Wahab *et al.*[19] using ADR (4 mg/kg twice/week × 2) demonstrated decreased cardiac GSH content and low SOD activity with elevated LPO in rat heart. Therefore, according to the present data and other reported studies, it seems that increased CK-MB and LDH in serum paralleled the inhibition of SOD activity, production of superoxide anion and TBARS in heart and liver of ADR treated rats. Thus, the present findings suggest that heart and liver damage resulting in leakage and increase in CK-MB and LDH levels in serum is a consequence of cellular oxidative injury induced by ADR mediated ROS production.

Because ADR results in free radical-mediated damage to cellular organelles and mammalian heart and liver that are relatively vulnerable to the toxic effects of FR, strategies to scavenge FR have shown to be promising. For this reason, identification and assessment of potential FR scavenging agents is of clinical interest. One group of candidate drugs in the –SH containing compounds. N-acetylcysteine, GSH and S-allylcysteine have been found to offer some cardioprotection in animal models of ADR cardiac and hepatictoxicity[21,25,36] respectively. Recently, it has been demonstrated that some –SH groups of CK, which have an important role in its enzyme activity, were very sensitive to ROS activated by ADR. This suggests that inactivation of CK in heart is due to oxidation of –SH groups at the active center of the enzyme.[33]

To the best of our knowledge, this is the first report utilizing MPG as a novel antioxidant agent when administered in combination with ADR to limit its FR-mediated tissue injury. The present study demonstrates that MPG greatly sustained the levels of GOT, GPT, CK-MB and LDH in

serum of rats receiving MPG for seven days before ADR treatment. This is accompanied by a marked protection against LPO as well as amelioration of the inhibition of SOD activity in heart and enhanced the activity of SOD in liver in the same treated rats. This is consistent with the finding that MPG strongly inhibited ascorbate induced LPO in rat liver microsomes.[37] Moreover, this implies that thiol compounds may have the ability to decrease LPO by scavenging the initiating and propagating radicals, which might be attributed to the high redox potential of MPG.[38] Because MPG is a small and diffusible molecule it can easily cross membranes and protect polyunsaturated fatty acids from FR mediated LPO by abstracting the hydrogen of the –SH group instead of methylene hydrogen of unsaturated lipids.[37]

Moreover, MPG may enhance endogenous antioxidative enzymes, as demonstrated in hepatic SOD (Table III), presumably by protecting essential –SH groups. The action of MPG in reducing TBARS in heart and liver is associated with increased SOD activity and significant amelioration of serum levels of GOT, GPT, CK-MB and LDH in rats receiving MPG before ADR. This result lends credence to the finding that postischemic myocardial dysfunction in canine myocardium has been reduced by sulfhydryl-containing FR scavenger MPG (4 mg/kg) and captopril (3 mg/kg, i.v.), a sulfhydryl containing angiotensin converting enzyme inhibitor, given before reperfusion.[39]

The control of intracellular iron concentration during ADR treatment seems to be important. In addition, the antiperoxidative effect of SH-compound like MPG may depend on its ability to bind metal ions. The affinity of metals like iron for reaction with sulfhydryl groups appear to be another possible mechanism for MPG induced detoxification to prevent formation of free radical generating compound, ADR-iron complex. This interpretation is in harmony with the concept that doxorubicin-induced myocardial toxicity can be prevented by the iron chelator desferrioxamine[11] and by Dexrazoxane[28] in rat. Furthermore, MPG prevents formation of ferryl myoglobin, which is capable of inducing peroxidative damage to membrane myocytes.[40] Thus, MPG might behave as oxyradical scavenger and inhibitor of ADR-iron complex formation, thereby contributing to protection against the ADR-induced oxidative toxicity.

Since GSH content in both tissues remained almost constant, as observed in this study under the present experimental condition, the mechanism of MPG action needs further investigation. However, the mechanism of MPG action most probably includes SH/S–S interchange reaction and FR scavenging ability.[8] In conclusion, MPG has a protective impact on ADR induced cytotoxicity and thus might serve as a novel co-therapeutic agent when administered in combination withADR to limit its oxidative organ injury.

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