

LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN ISOPROTERENOL INDUCED OXIDATIVE STRESS IN RAT ERYTHROCYTES

N. RATHORE, M. KALE, S. JOHN AND D. BHATNAGAR*

*School of Biochemistry,
D. A. University,*

Khandwa Road, Indore - 452 017

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Abstract : Isoproterenol, upon oxidation, produces quinones which react with oxygen to produce superoxide anions ($O_2^{\cdot-}$) and H_2O_2 . In the present study, isoproterenol was administered to rats in two doses so as to evaluate its β adrenergic and toxicological action in terms of lipid peroxidation (LPO) and antioxidant enzymes in erythrocytes. Isoproterenol (30 mg/100 g body wt.) was administered to rats and the animals were followed up to 7 days after administration. Some of these animals were treated with a second dose of isoproterenol 24 h after the first dose and the animals were followed up to 12 h. The result showed increased lipid peroxidation (LPO) and superoxide dismutase (SOD) activity in erythrocytes in response to isoproterenol. Catalase (CAT) activity in erythrocytes decreased with isoproterenol between day 2-7 as compared to control. The second injection of isoproterenol showed increased CAT activity in erythrocytes which decreased at 12 h as compared to control. The erythrocyte GSH content and glutathione-S-transferase (GST) activity decreased with isoproterenol treatment as compared to control. However, erythrocyte GSH content as well as GST activity both recovered towards control with time. Elevated serum lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and glutamate oxaloacetate transaminase (GOT) activity was observed after isoproterenol treatment. The results show increased LPO and altered antioxidant system in erythrocytes in response to isoproterenol induced oxidative stress.

Key words : isoproterenol catalase reactive oxygen species
lipid peroxidation superoxide dismutase
glutathione glutathione-S-transferase

INTRODUCTION

The autoxidation of catecholamines is slow at physiological pH. However, in presence of trace metals as well as alkaline

conditions, oxidation of catecholamines may increase manyfold (1). Catecholamines are useful under stress conditions but they may be toxic if produced or administered in high concentration due to generation of reactive

*Corresponding Author

oxygen species (ROS). Isoproterenol, a β adrenergic agonist has been shown to produce lipid peroxidative damage and alter the antioxidant defence system in heart, liver and kidney (2). However, there are no reports to show the effect of isoproterenol on erythrocytes. As the erythrocytes are prone to oxidative damage due to presence of polyunsaturated fatty acids (PUFA), heme iron and oxygen, isoproterenol may affect the erythrocytes adversely. However, the erythrocytes counteract the oxidative stress due to the presence of antioxidant enzymes such as Cu-Zn superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST) and glutathione reductase (GSH-R) which generates reduced glutathione (GSH) (3). In the present study, isoproterenol was administered to rats in two doses so as to evaluate the β adrenergic and the toxic action of isoproterenol. The study was undertaken to show whether isoproterenol produces lipid peroxidative damage and alters antioxidant system in erythrocytes.

METHODS

Female Wistar rats weighing 150-200 g were housed in polypropylene cages. The animals were maintained under standard conditions in the animal house and were allowed free access to drinking water and basal diet (Godrej, India). Isoproterenol (30 mg/100 g body wt.) was injected subcutaneously to rats and the animals were sacrificed after 6 h and 1, 2, 3, and 7 days of treatment. A second dose of isoproterenol was administered 24 hr after the first injection to rats and the animals were

followed up to 3, 6 and 12 h of second dose of isoproterenol.

The rats were sacrificed by decapitation and blood was collected by cardiac puncture in vials containing citrate (2%). Blood was centrifuged and the erythrocytes were washed twice with 0.1 M phosphate buffered saline (PBS, 1:9), pH 7.4. Packed cell volume (PCV) was adjusted to 5% with PBS, pH 7.4. Erythrocyte lysate was prepared according to the method of McCord and Fridovich (4) for the assay of antioxidant enzymes.

Lipid peroxidation (LPO) in erythrocytes was estimated by the method of Stocks and Dormandy (5) based on the thiobarbituric acid reactive substances (TBARS) reaction with malonyldialdehyde (MDA) formed owing to peroxidation of lipids.

Cu-Zn SOD activity was determined by the ability of the enzyme to inhibit the autoxidation of pyrogallol by the method of Marklund and Marklund (6). Catalase was assayed by the decomposition of H_2O_2 by the method of Aebi (7). Glutathione-S-transferase (GST) was estimated by the conjugation of the -SH groups with 1-chloro, 2, 4-dinitrobenzene (CDNB) by the method of Habig et al. (8). All enzyme assays were performed in duplicate. Glutathione content in blood was assayed using Ellman's reagent by the method of Beutler et al. (9).

The marker enzymes for myocardial infarction viz. creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and glutamate oxaloacetate transaminase (GOT) were estimated in serum by the kit method from Span Diagnostics Ltd., India.

The protein content was determined by the method of Lowry et al. (10). Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey multiple comparison procedure to calculate the significance. P values less than 5% were considered significant.

RESULTS

The animals treated with a single dose of isoproterenol showed increased MDA in erythrocytes up to day 1 of treatment (Table I). The MDA in erythrocytes, however,

remained high up to day 7 of isoproterenol treatment as compared to control. The animals treated with another dose of isoproterenol, 24 h after the first injection, also showed increased MDA in erythrocytes as compared to control.

Increased erythrocyte Cu-Zn SOD activity after isoproterenol treatment was observed at 6 h and between day 2-7 as compared to control (Table I). Another dose of isoproterenol, 24 h after the first injection, also showed increased erythrocyte SOD activity as compared to control.

TABLE I: Lipid peroxidation and activities of superoxide dismutase and catalase in erythrocytes after treatment with isoproterenol.

		LPO (n moles of MDA formed /ml PCV/hr)	SOD (units/mg protein)	CAT (n moles of H ₂ O ₂ decom- posed/mg protein/min)	GSH (μ moles of DTNB conjugated/ ml)	GST (n moles of CDNB conjugated/ mg protein)
Control		246±12	25.2±9.3	505±9	1.53±0.19	222±21
After 1 st injection of isoproterenol	6 h	319±14 (P<0.001)	43.5±1.1 (P<0.001)	581±67 (NS)	0.16±0.01 (P<0.001)	33.0±2.0 (P<0.001)
	1 day	341±7 (P<0.001)	29.2±1.7 (NS)	450±79 (NS)	0.88±0.09 (P<0.001)	18.1±0.7 (P<0.001)
	2 day	355±21 (P<0.001)	55.3±3.3 (P<0.001)	327±40 (P<0.001)	1.30±0.15 (P<0.05)	ND
	3 day	361±12 (P<0.001)	35.9±4.8 (P<0.01)	261±52 (P<0.001)	1.86±0.14 (P<0.01)	ND
	7 day	363±25 (P<0.001)	37.3±3.7 (P<0.01)	213±21 (P<0.001)	1.20±0.16 (P<0.01)	ND
After 2 nd injection of isoproterenol	3 h	322±53 [#] (P<0.001)	39.0±3.0** (P<0.001)	960±141*** (P<0.001)	0.70±0.14 [#] (P<0.001)	38.2±0.4*** (P<0.001)
	6 h	341±5 [#] (P<0.001)	34.2±3.3** (P<0.01)	540±62* (NS)	1.55±0.17** (NS)	78.3±1.5*** (P<0.001)
	12 h	296±8* (P<0.001)	35.5±0.6* (P<0.01)	375±66 [#] (P<0.01)	1.92±0.22*** (P<0.001)	153±1.7*** (P<0.001)

Values are mean ± SD of 6 animals in each experimental group.

Values given in parenthesis show significance as compared to 0 h control while

***P<0.001, **P<0.01, *P<0.05, # not significant, show significance as compared to day 1 after isoproterenol injection. ND = Not detected.

SC injection of isoproterenol (30 mg/100 g body wt.) was administered to rats and the animals were sacrificed at various intervals up to day 7 as shown above.

A second injection of isoproterenol was administered 24 h after the first injection and the animals were followed up to 12 h.

Catalase activity in erythrocytes decreased by day 2 of isoproterenol treatment and was significantly less than control up to day 7 (Table I). The second dose of isoproterenol, 24 h after the first injection, however, showed an increase in erythrocyte CAT activity at 3 h followed by a decrease in CAT activity by 12 h of second injection as compared to control.

The GSH content in erythrocytes decreased significantly after isoproterenol treatment except at day 3 which showed an increase in GSH content as compared to control (Table I). The second dose of isoproterenol 24 h after the first injection also decreased GSH content in erythrocytes which regained towards control by 6 h of second injection of isoproterenol.

The GST activity in erythrocytes decreased markedly within 6 h and was completely inhibited by day 2 of isoproterenol injection (Table I). The second injection of isoproterenol 24 h after the first dose also inhibited GST activity. However, mild recovery in erythrocyte GST activity was observed up to 12 h of second injection.

Serum lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and glutamate oxaloacetate transaminase (GOT) activity was significantly increased after isoproterenol treatment (Table II). SGOT activity recovered to control by day 3 of isoproterenol treatment. However, serum LDH and CPK activity decreased marginally by day 7 of isoproterenol treatment. After 12 h of second injection of isoproterenol, serum LDH, CPK and GOT activities were significantly elevated as compared to control.

TABLE II: Serum enzyme activities in rats after treatment with isoproterenol.

		LDH	CPK (IU/l)	SGOT
Control		254±15	114±10	62±5
After 1 st injection of isoproterenol	6 h	1161±26 (P<0.001)	395±14 (P<0.001)	85±10 (P<0.05)
	1 day	1142±37 (P<0.001)	447±29 (P<0.001)	142±27 (P<0.01)
	2 day	1122±83 (P<0.001)	366±14 (P<0.001)	86±11 (P<0.05)
	3 day	1159±13 (P<0.001)	418±9 (P<0.001)	61±4 (NS)
	7 day	1089±91 (P<0.001)	242±4 (P<0.001)	42±13 (NS)
After 2 nd injection of isoproterenol	12 h	1073±31 (P<0.001)	288±22 (P<0.001)	111±1 (P<0.001)

Values are mean ± SD of 4 animals in each experimental group.

Values given in parenthesis show significance as compared to control.

DISCUSSION

In the present study, two doses of isoproterenol were used to show β adrenergic and toxic action of isoproterenol. The dose was similar to that administered by other workers to induce myocardial infarction. (11-13). The treatment with isoproterenol produced oxidative stress and induced LPO in erythrocytes. LPO may be due to the oxidation of isoproterenol to semiquinone which reacts with oxygen with the production of superoxide anions ($O_2^{\bullet-}$) and H_2O_2 . Catecholamines readily form chelate complexes with metal ions such as iron, copper and manganese which strongly catalyse oxidation of catecholamines (14). It is suggested that oxidation of isoproterenol involves ROS which may cause peroxidative damage to erythrocytes.

The increase in erythrocyte SOD activity in isoproterenol treated animals may probably be an adaptive response towards oxidative stress. However, the gradual decrease in erythrocyte CAT activity after isoproterenol administration may be due to excessive generation of $O_2^{\bullet-}$ leading to the inactivation of the enzyme, as $O_2^{\bullet-}$ has been shown to reduce CAT activity (15). Ji et al. (16) reported similar results with reduced CAT activity at the onset of reperfusion without any decrease in SOD activity and suggested inactivation of the enzyme due to superoxide anions.

The isoproterenol treatment showed depletion of erythrocyte GSH up to day 1. However, the GSH content recovered towards control over a time period. The quinone toxicity is mainly attributed to their interaction with cellular -SH groups with the formation of stable adducts (17). Aminochromes due to the presence of

indoline quinone ring interact with nucleophiles of the cells, particularly acid soluble thiols such as cysteine, glutathione and protein -SH groups to form reduced substituted thiol addition products. The incubation of blood with adrenaline was shown to induce a decrease in GSH content of erythrocytes due to reactions involving adrenaline oxidation products (18). The mild recovery of erythrocyte GSH content as compared to control after day 2 of isoproterenol administration may probably be an important antioxidant function to diminish quinone toxicity. The decrease in erythrocyte GST activity resulting in its complete inhibition may partially be responsible for increased erythrocyte LPO after a dose of isoproterenol. The depletion of erythrocyte GSH content may also lower the GST activity as GSH is required as substrate for GST activity. The mild recovery in GSH content in erythrocytes may restore erythrocyte GST activity towards control.

The diagnostic enzymes of clinical importance such as serum LDH, CPK and GOT increased with isoproterenol treatment. Isoproterenol is known to cause micro-infarction (19) and it may increase serum enzymes. However, recovery from myocardial infarction within 7 days of isoproterenol treatment may show reversal of serum GOT activity towards control. It is concluded that semiquinone free radical intermediates from isoproterenol induces LPO and alters the antioxidant system in erythrocytes to counteract isoproterenol induced oxidative stress.

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