Corrective Effect of Clotrimazole and β-Ionol during Exposure to Thiophenol

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Toxic effect of thiophenol on rats can be prevented by injection of antioxidant β-ionol and clotrimazole, an inductor of phase I and II detoxication enzymes. Both agents increase O-demethylase activity of cytochrome P-450, but do not prevent inhibition of its water-soluble form, and activate cytosol and microsomal glutathione-dependent enzymes. Both agents protected erythrocytes from peroxide damage by thiophenol and simultaneously enhanced its prooxidant effect in the liver.

Key Words: *correction; intoxication; clotrimazole; β-ionol; glutathione-dependent enzymes; cytochrome P-450*

Aromatic thiols and their disulfides exert a potent destructive effect on erythrocyte membrane and hemoglobin [1,13] and a pronounced hepatotropic effect. Wide use of aromatic heterocyclic disulfides in industry attracts attention to the possibility of drug correction of the probable pathological conditions caused by these substances. We investigated potential corrective drugs clotrimazole, an inductor of liver detoxication enzyme systems (cytochrome P-450 and conjugation enzymes) and β-ionol, an inductor of these systems and effective antioxidant [6,13].

MATERIALS AND METHODS

Experiments were carried out on random-bred male albino rats from Rappolovo breeding canter. The animals were divided into 4 groups, 16 rats each. Group 1 were intact rats, animals of groups 2-4 were intragastrically given thiophenol in a single dose of 200 mg/kg (LD_{50}). Groups 3 and 4 rats were intragastrically treated with clotrimazole or β-ionol in daily doses of 75 and 600 mg/kg, respectively, [2,6,13] in vegetable oil 2 days before thiophenol injection. Groups 1 and 2 rats (control) were fed vegetable oil for 2 days. The animals were decapitated under ether narcosis 24 h after thiophenol injections.

Liver microsomes were obtained by differentiated centrifugation of liver homogenates as described previously [5]. Cytochrome P-450 activity (liver microsomes) was evaluated by the reaction with *n*-nitroanisole (O-demethylase activity) and amaranthe (reducing activity) as described elsewhere [9]. Glutathione transferase (GT) activity was evaluated using 2,4-dinitrochlorobenzene [11], and activities of antioxidant defense enzymes glutathione reductase (GR) and catalase [10] were measured in the liver cytosol and microsomes and in erythrocyte lysate. The intensity of free-radical oxidation (FRO) in liver microsomes was evaluated by the concentration of malonic dialdehyde (MDA) forming during ascorbate-dependent lipid peroxidation after 20-min incubation [4]. Antioxidant activity in erythrocyte membranes and plasma was evaluated from the intensity of FRO by chemiluminescence (CL) induced by Fenten mixture and measured by BKhL-06 biochemiluminometer. The intensity of the process was evaluated by slow flash photosum. Protein was measured by the Lowry method [12], blood hemoglobin by the cyanide method [3]. The results were statistically processed using Student's *t* test.

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RESULTS

Injection of thiophenol destroyed hemoglobin and almost twofold suppressed activities of glutathione-dependent enzymes GT and GR in erythrocytes (Table 1), which was due to its prooxidant effect and, hence, oxidative destruction of these enzymes. Thiophenol stimulated FRO in erythrocyte membranes and blood plasma. Pretreatment with β-ionol exerted a protective effect: it inhibited CL in erythrocyte membranes and blood plasma, restored GR activity by 80% and GT activity almost completely. Clotrimazole also restored GR activity and decreased erythrocyte membrane and plasma CL, but did not reactivate GT.

Thiophenol reduced activity of water-exposed fraction of cytochrome P-450 (amaranthe substrate) (Table 2), which can explain its allergenic effect. Thiophenol intensified FRO in liver microsomes, causing a 2-fold increase in catalase activities both in liver microsomes and cytosol (Table 2). Pretreatment with clotrimazole and β-ionol stimulated O-demethylase activity of cytochrome P-450, but did not prevent water-exposed enzyme from inhibition with thiophenol (apparently did not induce the enzyme). Both drugs activated cytosol and microsomal glutathione-dependent enzymes, the effect of β-ionol being more potent (GT activity increased almost 3-fold and catalase activity did not differ from the control).

Surprisingly, combined administration of thiophenol and corrective agents produced a pronounced prooxidant effect in liver microsomes, though both drugs, primarily β-ionol, were antioxidants. This can be due to considerable activation of cytochrome P-450 (induction) by these agents or their metabolites [7], which is known to be associated with the production of active oxygen forms and FRO activation [8]. On the other hand,

TABLE 1. Antioxidant Activity in Erythrocytes (*M±m*)

Parameter	Intact	Thiophenol		
		control	+clotrimazole	+β-ionol
Activity per mg Hb				
GT, nmol/min	4.3±0.4	2.7±0.2*	2.6±0.1*	3.5±0.2
GR, nmol/min	1127.7±61.4	587.4±42.7*	924.8±109.1	1062.5±72.4
catalase, μmol/min	1802.1±98.0	1387.0±146.6	1388.4±259.8	1585.3±85.2
CL, 10^2 cpm/mg protein				
erythrocyte membranes	70.8±11.8	89.3±5.8	52.0±4.9*	34.1±17.8*
serum	65.1±12.6	75.4±11.4	37.9±4.8*	40.2±5.4*

Note. Here and in Table 2: *$p<0.05$ compared to intact animals.

TABLE 2. Activities of Detoxication Enzymes and Antioxidant Activity in Hepatocytes (*M±m*)

Activity per mg protein/min	Intact	Thiophenol		
		control	+clotrimazole	+β-ionol
GT, nmol				
cytosol	541.9±36.4	662.5±60.7	1034.9±129.5	1374.3±66.5*
microsomes	201.6±19.0	208.2±25.7	337.8±41.2	529.5±35.3*
GR, nmol				
cytosol	66.1±3.2	80.7±12.7	120.8±4.2*	132.7±5.2*
microsomes	126.2±15.3	131.9±15.6	193.5±8.4	219.6±11.7*
Catalase, μmol				
cytosol	197.4±14.6	330.0±24.3*	218.6±27.3	137.9±9.8*
microsomes	390.2±59.1	640.0±36.3*	403.9±51.8	263.6±24.9
Cytochrome P-450 (microsomes), nmol				
n-nitroanisole	4.2±0.9	6.1±0.2	12.0±2.9*	18.7±2.5*
amaranthe	2.0±0.2	1.2±0.2*	1.0±0.1*	1.0±0.1*
MDA (microsomes), nmol	51.5±3.8	86.4±15.8*	590.5±212.6*	545.0±70.3*

isolated administration of clotrimazole or β-ionol exerted no prooxidant effect, which was confirmed by our experiments [1] and published reports [4,6]. High survival rate in animals treated with β-ionol and clotrimazole (100% and 70%, respectively) indicates that intensification of LPO in hepatocytes is not the key damaging factor because the primary target for aromatic thiols are blood erythrocytes [13]. Their protection from peroxide damage determines the total protective effect of the drugs.

Our findings suggest that of the two agents stimulating the detoxication systems antioxidant β-ionol produces a more potent protective effect in acute poisoning with thiophenol. However, if the corrective drug acts as cytochrome P-450 inductor (clotrimazole or β-ionol in a high (600 mg/kg) concentration), its combination with the toxic agent can exert a prooxidant effect.

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