Inhibition of Cytochrome P-450 with 2-Diethylaminoethyl-2,2-Diphenylpropylacetate (SKF-525A) Reduces Immunotoxicity of Chlorinated Carbohydrates

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Experiments on outbred albino rats showed that single intraperitoneal injection of cytochrome P-450 inhibitor 2-diethylaminoethyl-2,2-diphenylpropylacetate (SKF-525A) in a dose of 50 mg/kg before acute poisoning with 1,2-dichloroethane and trichloroethane in a dose of 1.0 LD_{50}, metabolized in the body to compounds with higher toxicity (the phenomenon of "lethal synthesis") reduced their immunotoxicity by decreasing the formation of their biotransformation products.

Key Words: *SKF-525A; cytochrome P-450; 1,2-dichloroethane; trichloroethane; immunotoxicity*

Many xenobiotics (for example, barbiturates) potentiate the immunotoxic effects of chemicals forming highly toxic biotransformation products ("lethal synthesis") by inducing the system of cytochrome P-450-dependent monooxygenases (monooxygenase system) [1,3,5,9]. However, the effect of cytochrome P-450 inhibitors on the immunotoxic characteristics of compounds metabolized in the body to highly toxic products is little studied [9,12]. Among reversible and irreversible inhibitors of cytochrome P-450 are numerous compounds of different chemical nature (esters, alcohols, phenols, benzene derivatives, hydrasines, SKF-525A, polyhalogenated alkanes, protein synthesis inhibitors, *etc.*) [1,11,12].

Published data suggest that pretreatment with 2-diethylaminoethyl-2,2-diphenylpropylacetate (SKF-525A), a reversible inhibitor of cytochrome P-450, reduces the immunotoxic effects of chemicals forming highly toxic metabolites of predominantly monooxygenase system, *e.g.* 1,2-dichloroethane (DCE, phosphamide, parathion, trichloroethylene (TCE), acrylonitrile, *etc.* [1,9,11,12].

We evaluated damage to the immune system caused by toxicants metabolized by the monooxygenase system to highly toxic compounds (DCE and TCE) after injection of cytochrome P-450 inhibitor SKF-525A and its relationship with the content of DCE metabolites in the spleen.

MATERIALS AND METHODS

Experiments were carried out on outbred albino rats of both genders (180-250 g). Toxicants DCE and TCE are chlorinated carbohydrates; they are frequent causes of acute poisoning associated with high mortality [6-8]. The toxicants were administered orally in a dose of LD_{50}. The mean lethal doses of DCE and TCE for rats were 0.93±0.11 and 4.7±0.4 g/kg, respectively. SKF-525A was injected intraperitoneally in a dose of 50 mg/kg 1 day before administration of chlorinated carbohydrates.

Humoral immune response to thymus-dependent (sheep erythrocytes) and independent (typhoid Vi antigen — Vi-Ag) antigens was evaluated after 5 days by the number of antibody-producing

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cells in the spleen after acute intoxication with the chemical toxicants with simultaneous intraperitoneal immunization of rats with these antigens in doses of 2×10^8 cells and 8 μg/kg, respectively. Humoral immune response to sheep erythrocytes in the test used in our study characterized the capacity of type 1 T-helpers to participate in the production of IgM by B-lymphocytes (plasma cells), while humoral immune response to Vi-Ag reflects activity of IgM synthesis by these cells without participation of type 1 T-helpers [4]. Activity of natural killers was evaluated spectrophotometrically by the parameter of natural cytotoxicity 48 h after acute poisoning. Antibody-dependent cytotoxicity was evaluated spectrophotometrically 5 days after immunization with 10^8 sheep erythrocytes using sheep splenocytes [3]. The formation of delayed-type hypersensitivity was evaluated by the increment in the hind paw weight (in percents). The rats were intraperitoneally immunized with 10^8 sheep erythrocytes 30 min after injection of toxic chemicals. The resolving dose of sheep erythrocytes (5×10^8) was injected under the hind paw aponeurosis after 4 days. Delayed-type hypersensitivity was evaluated after 24 h [3,4].

The content of DCE biotransformation products in the spleen was evaluated 24 h after intoxication on an LCM-8MD chromatograph with plasma ionization detector [2,8].

The data were processed using Student's *t* test.

RESULTS

Humoral immune response to thymus-dependent and independent antigens, activity of natural killer cells, antibody-dependent cell cytotoxicity, and delayed-type hypersensitivity reaction considerably decreased after acute intoxication with DCE (by 2.02, 1.66, 1.78, 2.11, and 1.99 times, respectively; $p<0.05$; Table 1) and TCE (by 1.86, 1.49, 1.47, 1.88, and 1.82 times, respectively; $p<0.05$). The immunosuppressive effects of chlorinated carbohydrates decreased significantly ($p<0.05$) after pretreatment with SKF-525A. Acute DCE poisoning after injection of SKF-525 was followed by increased thymus-dependent and independent humoral immune response, natural cytotoxicity, antibody-dependent cell cytotoxicity, and formation of delayed-type hypersensitivity (by 1.44, 1.33, 1.39, 1.49, and 1.47 times, respectively) in comparison with the corresponding parameters after intoxication. All values remained significantly below the control ($p<0.05$). Injection of SKF-525A before DCE intoxication produced a less pronounced immunoprotective effect, presumably because of higher immunotoxicity of DCE.

The content of DCE metabolites (2-chloroethanol, 2-chloroacetaldehyde, and chloroacetic acid) in the spleen was measured 24 h after DCE intoxication. Injection of SKF-525A before DCE decreased the concentrations of 2-chloroethanol and chloroacetic acid by 1.91 and 1.65 times, respectively ($p<0.05$), no 2-chloroacetaldehyde was detected in the organ (Table 2). This suggests that reduction of chlorinated carbohydrates immunotoxicity after SKF-525A pretreatment was associated with inhibition of DCE and TCE metabolism by P-450-dependent monooxygenases.

It seems that chlorinated carbohydrates can undergo biotransformation not only in the liver, but also in lymphocytes. It was proven, for example, that vitamin A, levamisole, phenobarbital, and other substances can induce cytochrome P-450 in T cells and natural killer cells and stimulate their activities [10].

Inhibition of the monooxygenase system of the liver and lymphoid tissue with SKF-525A significantly reduced biotransformation of chlorinated carbohydrates and inhibited the formation of compounds more toxic than the injected chemicals [3,9]. This was paralleled by reduction of the immunosuppressive effects of DCE and TCE.

TABLE 1. Effects of Chlorinated Carbohydrates and SKF-525A after Acute Intoxication with DCE and TCE on Immunity Parameters in Rats (n=7-11, $M\pm m$)

Group	APC to sheep erythrocytes in spleen ($\times10^3$)	APC to Vi-Ag in spleen ($\times10^3$)	Natural cytotoxicity, %	Antibody-dependent cellular cytotoxicity, %	Delayed-type hypersensitivity, %
Control	37.7±3.0	28.4±2.3	34.3±1.9	13.7±1.4	35.4±2.9
DCE	18.7± 2.2*	17.1±1.5*	19.3±1.6*	6.5±1.1*	17.8±2.0*
TCE	20.3±2.1*	19.0±1.6*	22.0±1.7*	7.3±1.2*	19.4±1.9*
SKF-525A+DCE	27.0±2.3*+	22.8±1.9*+	26.9±1.8*+	9.5±1.3*+	26.1±2.5*+
SKF-525A+TCE	29.3±3.1x	24.7±2.0x	27.3±2.0*x	10.2±1.5x	30.2±2.6x

Note. $p<0.05$ compared to *control, +DCE, xTCE.

TABLE 2. Effects of DCE and SKF-525A 24 Hours after Acute Intoxication with DCE (after 24 h) on the Content of DCE Metabolites in Rat Spleen (mg/kg; $M \pm m$; n=7-11)

Metabolite	DCE	SKF-525A+DCE
2-Chloroethanol	0.21±0.03	0.11±0.03*
2-Chloracetaldehyde	0.12±0.02	Not measured
Chloroacetic acid	2.03±0.24	1.23±0.13*

Note. *p<0.05 compared to the control.

Hence, the use of cytochrome P-450 inhibitor SKF-525A, before acute intoxication of albino rats with DCE and TCE in LD_{50} reduced their immunotoxic effects due to inhibition of the formation of their toxic biotransformation products.

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