PHARMACOLOGY AND TOXICOLOGY

Effects of Sex Steroid Hormones on Lipid Peroxidation and Glutathione Antioxidant System in Rat Skin

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Effects of estradiol and testosterone on the intensity of lipid peroxidation and contents of glutathione redox system components in the dermis and epidermis of rat skin were studied. Only estradiol induced considerable dose-dependent and tissue-specific biphasic antioxidant effects on the skin.

Key Words: *skin; dermis; epidermis; estradiol; lipid peroxidation; chemiluminescence; glutathione*

Lipid peroxidation (LPO) typical of all tissues and regulating cell proliferation, lipid-protein interactions, and membrane permeability [1] also affects cell membranes and their functions. The intensity of LPO is regulated by systems of free radical generation and antioxidant protection.

LPO processes in the blood are activated in some skin diseases [4]. The pathogenesis and severity of dermatoses depend on the sex of a patient [8], which can be explained by the effects of sex hormones on the degree of lipid oxidation in the skin.

The glutathione redox system is one of the most important antioxidant systems in the body [3,6]. However, we found no publications concerning activity of this system in the skin of healthy humans and patients with dermatoses. Some steroid hormones possess antioxidant properties [1,7], but their effects on activity of LPO and glutathione redox system in the skin are still unknown.

Here we studied the effects of estradiol and testosterone on LPO and glutathione redox systems in the dermis and epidermis of intact rats.

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MATERIALS AND METHODS

Experiments were performed on adult male and female (in the diestrus phase [5]) rats weighing 140-180 g. Each series was conducted on 6-9 animals. The rats were killed under ether anesthesia 5, 30, 60, and 90 min and 1, 2, 3, 6, 12, and 24 h after injection of hormones. Skin homogenates were prepared by the routine method [11]. The amplitude of slow flash of Fe^{2+}-induced chemiluminescence (H_{SF}) and contents of thiobarbituric acid reactive substances (TBA)-reactive substances in the dermis and epidermis were determined as described previously [2,9]. The contents of reduced glutathione (GSH), glutathione reductase (GR), and glutathione peroxidase (GPx) were estimated spectrophotometrically [3]. Hormones were injected subcutaneously in doses of 1 and 10 mg/kg in 1 ml 20% ethanol. Protein concentration was measured by the method of Lowry [13]. The results were analyzed by Student's *t* test.

RESULTS

Series I showed that the amplitude of slow flash of Fe^{2+}-induced chemiluminescence (H_{SF}) and the content of TBA-reactive substances in the dermis and epider-

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mis depended on the rate of free radical generation and their involvement in tissue metabolism.

All parameters of LPO in the dermis of intact rats surpassed those in the epidermis; they were also higher in male rats than in females. The data suggest that the intensity of LPO in rat skin depends on the sex (i.e., on the content of sex hormones) and type of the tissue (Table 1).

Ovariectomy (but not orchiectomy) activated LPO in rat skin (Table 1), changes in H_{SF} were more pronounced than accumulation of TBA-reactive substance, especially in the dermis. These differences disappeared after administration of estradiol. Hence, the intensity of oxidative processes in the skin is regulated primarily by estrogens.

The intensity of free radical generation and H_{SF} decreased 30 min after subcutaneous injection of 1 mg/kg estradiol. The content of TBA-reactive substance decreased by 20-40% depending on the type of tissue and gender; the most pronounced changes were observed in the skin of male rats (Table 1). Testosterone induced no statistically significant changes in the intensity of LPO in the dermis and epidermis of male and female animals.

In a dose of 10 mg/kg estradiol produced a more pronounced inhibitory effect on LPO in the dermis and epidermis, especially in male rats; H_{SF} and the content of TBA-reactive substance decreased by 25-63 and 35-50%, respectively. Testosterone in a dose of 10 mg/kg practically did not change these parameters (Table 1).

Subcutaneous injection of 10 mg/kg estradiol reduced the intensity of LPO, which attained its minimum 1 h postinjection in the epidermis (45% of the control), but then increased and peaked 3 h postinjection (39% of the control) in the dermis of male rats. It can be assumed that estradiol being a true antioxidant [7] initially decreased the content of free radicals in the skin and inhibited their generation, but then (2 h postinjection) some other mechanisms appeared to be involved in these processes. Apart from its direct effect on free radicals, estradiol can trigger the phosphoinositol cycle in cell membranes, which activates NADPH oxidase and stimulates generation of reactive oxygen species [2,10], and the arachidonic cycle [10] contributing to the accumulation of oxygen and lipid radicals.

H_{SF} in the dermis and epidermis of male and female animals decreased by 10-20% 90 min after injection of 10 mg/kg testosterone and then returned to the control level.

In series II, we studied the effects of estradiol and testosterone on the glutathione redox system (content of GSH and activities of GR and GPx) in the skin of intact rats. The content of GSH and activities of GR and GPx were higher in females than in males, and in

TABLE 1. Effects of Estradiol and Testosterone on LPO in the Skin of Intact Rats 30 min Postinjection (*M±m*, 6-9 Observations)

Factor		Females		Males	
		epidermis	dermis	epidermis	dermis
Amplitude of slow flash of chemiluminescence, arb. units					
Control		1.2±0.05^{xx}	1.6±0.06^{x,xx}	1.6±0.05	2.3±0.08^{x}
Ovariectomy		1.8±0.07*	3.0±0.08^{*x}		
Estradiol	1 mg/kg	0.95±0.1	1.3±0.2	1.0±0.1*	1.8±0.1^{*x}
	10 mg/kg	0.90±0.1	0.9±0.2*	0.6±0.1*	1.0±0.1^{*x}
Orchiectomy				1.7±0.08	2.4±0.1^{x}
Testosterone	1 mg/kg	1.0±0.1	1.5±0.3	1.3±0.4	2.0±0.5
	10 mg/kg	0.9±0.2	1.4±0.3	0.7±0.3	1.3±0.1*
Content of TBA-reactive substance, nmol/mg					
Control		1.1±0.03	1.7±0.04^{x}	1.3±0.2	2.0±0.2
Ovariectomy				1.6±0.09*	2.1±0.03^{*x}
Estradiol	1 mg/kg	0.8±0.1*	1.2±0.1^{*x}	1.0±0.1*	1.1±0.2*
	10 mg/kg	0.7±0.06*	1.0±0.1^{*,x}	0.8±0.1*	1.0±0.08^{*x}
Orchiectomy				1.6±0.2	2.2±0.3
Testosterone	1 mg/kg	1.0±0.2	1.7±0.3^{x}	1.1±0.2	1.6±0.3
	10 mg/kg	1.0±0.07	1.4±0.2^{x}	1.2±0.2	1.4±0.4

Note. $^{*}p<0.05$ compared with the control and $^{x}p<0.05$ compared with the epidermis.

TABLE 2. Effects of Estradiol and Testosterone on Glutathione Redox System in Rat Skin 30 min Postinjection (*M±m*)

Factor		Tissue	Redox system parameters		
			GSH, nmol/mg protein	activity, μmol/min/mg protein	
				GR	GPx
Females					
Control		Epidermis	3.10±0.25	0.019±0.002	0.034±0.001
		Dermis	3.70±0.20	0.022±0.002	0.037±0.001
Ovariectomy		Epidermis	2.70±0.10*	0.011±0.001*	0.027±0.001*
		Dermis	3.20±0.12*	0.016±0.001*	0.030±0.001*
Estradiol	1 mg/kg	Epidermis	3.20±0.20	0.021±0.0018	0.035±0.0020
		Dermis	3.90±0.15	0.023±0.0020	0.037±0.0018
	10 mg/kg	Epidermis	3.75±0.12*	0.030±0.0005*	0.043±0.001*
		Dermis	4.50±0.10*	0.032±0.001*	0.040±0.001*
Testosterone	1 mg/kg	Epidermis	3.00±0.40	0.020±0.0020	0.035±0.0015
		Dermis	3.72±0.30	0.025±0.0025	0.040±0.001
	10 mg/kg	Epidermis	3.00±0.40	0.025±0.0020	0.033±0.002
		Dermis	3.75±0.20	0.023±0.0020	0.040±0.002
Males					
Control		Epidermis	2.80±0.19	0.017±0.0016	0.030±0.0011
		Dermis	3.30±0.12	0.020±0.0012	0.034±0.0030
Orchiectomy		Epidermis	2.78±0.20	0.018±0.0020	0.028±0.0020
		Dermis	3.20±0.25	0.021±0.0015	0.035±0.0025
Estradiol	1 mg/kg	Epidermis	3.05±0.20	0.020±0.0020	0.033±0.0020
		Dermis	3.50±0.15	0.022±0.0030	0.038±0.0020
	10 mg/kg	Epidermis	4.40±0.10*	0.028±0.0007*	0.040±0.0005*
		Dermis	3.50±0.10*	0.035±0.0010*	0.041±0.001*
Testosterone	1 mg/kg	Epidermis	3.00±0.40	0.019±0.002	0.030±0.0020
		Dermis	3.32±0.30	0.025±0.0035	0.035±0.0030
	10 mg/kg	Epidermis	3.26±0.40*	0.028±0.0030	0.035±0.0020
		Dermis	3.35±0.50	0.028±0.0025	0.035±0.0030

Note. *$p<0.05$ compared with the control.

the dermis than in the epidermis (Table 2). However, these differences were not statistically significant (except for different contents of glutathione in male dermis and epidermis). Relatively high activity of the glutathione redox system in the dermis is probably due the presence of free radical-generating macrophages and neutrophils [2].

All parameters of the glutathione redox system decreased after ovariectomy (but not after orchiectomy) and returned to the control levels after estradiol replacement therapy (Table 2).

The content of GSH and activities of GR and GPx in the skin dose-dependently increased 30 min after injection of 1 and 10 mg/kg estradiol (Table 2), but did not change after administration of testosterone in the same doses.

It can be assumed that the antioxidant properties of estrogens are associated with their effects not only on the content of free radicals and lipid peroxides in cells, but also on tissue glutathione redox systems. Activation of these enzymes provide potent antioxidant effects.

Our findings indicate that estrogens maintain metabolic processes and protect the skin from free radicals, whose content increases with age. Therefore, they should be used for replacement therapy in women with ovarian hypofunction.

Time-dependent effects of estrogens on LPO in the skin should be considered during hormone replacement therapy. Antioxidant effects of estrogens rapidly disappear, and the intensity of LPO processes then increases (rebound effect). Therefore, the prepa-

rations characterized by sustained release of estrogens (transdermal plasters and storage preparations) are more appropriate than peroral estrogens characterized by peak concentrations and rapid elimination from the body.

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