Phospholipid Composition of Granulation and Fibrous Tissues in Rats after Local Application of Melatonin

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We studied the effect of melatonin applied locally in doses of 1.5 and 15 mg/ml on the phospholipid composition of granulation and fibrous tissues in rats at various stages of regeneration. Melatonin (especially in a dose of 1.5 mg/ml) increased the total phospholipid content on day 8 of regeneration, which was primarily related to accumulation of ethanolamine- and serine-containing fractions. Melatonin in a dose of 15 mg/ml increased the concentration of choline-containing fractions on day 5 of regeneration.

Key Words: *granulation-and-fibrous tissue; melatonin; phospholipids*

Phospholipids perform important physiological and biochemical functions in posttraumatic regeneration [6]. However, little attention was given to changes in the phospholipid composition of blood plasma and exudate during wound healing [9]. The effects of melatonin in various doses on the phospholipid composition of the granulation and fibrous tissues are poorly known. Our previous studies showed that local application of melatonin decreases the content of total lipids and their fractions in the granulation and fibrous tissues on day 5 of regeneration [3]. In a dose of 1.5 mg/kg this hormone produces a triglyceride-lipase effect. Treatment with melatonin in a dose of 15 mg/kg produces similar changes on days 5 and 8 of maturation of the granulation and fibrous tissue. The shift in metabolic transformations toward the transacylase mechanism produced by melatonin is not accompanied by an increase in the level of free fatty acids. Local application of melatonin decelerates healing of skin wounds due to inhibition of repair processes. Melatonin induces changes in the spectrum of water-soluble proteins, glycosaminoglycans, and lipids in the granulation tissue [4].

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Here we studied the effects of melatonin applied locally in various doses on the phospholipid composition of the granulation and fibrous tissues in rats.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200-250 g. Tissue samples was obtained by the method described elsewhere [7]. Biological samples were taken 5 and 8 days after surgery. A cellophane film was placed on the wound surface. Melatonin in doses of 1.5 and 15 mg/ml (0.1 ml) was applied to this film for 5 or 8 days. Control animals were treated with 0.1 ml distilled water [3].

The contents of total phospholipids (TPL) and their fractions were measured in the granulation tissues. Lipids were extracted by the method of Folch [10]. Lipid fractions were separated by microthin-layer chromatography on Silica gel [1]. TPL and the absolute and relative contents of phospholipid fractions glycerophosphates (GLP), lysophospholipids (LPL), sphingomyelins (SPM), phosphatidylcholines (PC), phosphatidylinositols and phosphatidylserines (PI+PS), phosphatidylethanolamines (PEA), and phosphatidic acids and polyglycerophosphates (PA+PGP) were measured.

The results were analyzed by Student's *t* test [5].

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TABLE 1. Effects of Local Application of Melatonin on the Contents of TPL and Their Fractions in Granulation-and-Fibrous Tissue in Rats (mg/100 g wet weight, $M \pm m$, n=6-10)

Phospholipid fractions		Day 5 of regeneration			Day 8 of regeneration		
		control	melatonin, mg/ml		control	melatonin, mg/ml	
			1.5	15		1.5	15
TPL		315.0±25.2	387.0±27.1	455.0±40.9*	219.0±17.5	585.0±46.8$^+$	380.0±26.6$^+$
GLP	abs.	22.3±1.8	39.1±2.3*	31.4±2.7*	21.0±2.0	33.3±2.9$^+$	43.4±3.0$^+$
	%$^+$	7.2±0.5	10.1±0.6*	6.9±0.6	9.6±0.9	5.7±0.5$^+$	11.4±0.8
LPL	abs.	44.7±3.1	40.6±2.7	50.4±3.6	20.4±1.1	46.2±3.5$^+$	50.6±3.6$^+$
	%$^+$	14.2±1.0	10.5±0.7*	11.1±0.7*	9.3±0.5	7.9±0.6	13.3±1.1$^+$
SPM	abs.	29.9±2.5	53.8±4.6*	67.7±5.9*	22.5±2.0	77.2±6.4$^+$	59.0±5.3$^+$
	%$^+$	9.5±0.8	13.9±1.2*	14.9±1.3*	10.3±0.9	13.2±1.1	15.5±1.4$^+$
PC	abs.	117.0±9.4	54.6±3.9*	146.0±14.1	25.4±2.0	75.4±7.5$^+$	692.0±6.1$^+$
	%$^+$	37.0±3.0	14.1±10*	32.1±3.1	11.6±0.9	12.9±1.3	18.2±1.6$^+$
PI+PS	abs.	10.4±0.8	57.1±5.0*	39.5±3.2*	33.3±2.6	99.0±8.3$^+$	52.6±4.4$^+$
	%$^+$	3.3±0.1	14.9±1.3*	8.7±0.7	15.2±1.2	16.6±1.4	13.3±1.1
PEA	abs.	56.7±4.1	87.1±6.2*	75.4±5.3*	56.7±5.1	158±9.9$^+$	48.7±4.2
	%$^+$	18.0±1.3	22.5±1.6	16.6±1.5	25.9±1.8	27.1±1.7	12.8±1.1$^+$
PA+PGP	abs.	34.0±2.2	54.6±3.9*	44.1±3.6*	39.6±3.3	95.0±8.0$^+$	57.0±4.4$^+$
	%$^+$	10.8±0.7	14.0±1.0*	9.7±0.8	18.1±1.5	16.6±1.4	15.5±1.2

Note. *p<0.05 compared to the control at the same stage of regeneration; $^+$percent of TPL.

RESULTS

The amount of TPL remained unchanged in control animals. Melatonin in a dose of 1.5 mg/ml slightly increased TPL content in the granulation tissue on day 5 of regeneration (p>0.05). These changes were primarily related to an increase in the absolute and relative contents of most phospholipid fractions (Table 1). However, the concentration of PC decreased. Melatonin in a dose of 1.5 mg/ml more significantly increased TPL content on day 8 of regeneration (by 2.7 times). The absolute content of most phospholipid fractions increased, while their relative content remained unchanged. After application of 15 mg/ml melatonin TPL content increased by 1.4 and 1.7 times on days 5 and 8 of regeneration, respectively. The absolute content of phospholipid fractions remained high (Table 1). On day 5 of regeneration the relative content of LPL decreased, while the content of SPM increased. On day 8 of regeneration the relative content of PEA decreased, while the relative and absolute contents of most phospholipid fractions tended to increase.

Our results suggest that local application of melatonin (particularly in the lower dose) increased the content of TPL in the granulation tissue on day 8 of regeneration. These changes were primarily related to accumulation of PEA, PI+PS, and SPM. Melatonin in a dose of 15 mg/ml increased the content of choline-containing fractions at the early stage of regeneration and equalized the contents of most phospholipid fractions at the late stages of regeneration. These data confirm the notion that phospholipids play an important role in regeneration. Our findings indicate that various humoral factors, including melatonin, regulate metabolism of phospholipids in regenerating tissues [9]. Changes in metabolic relationships between various phospholipids play a role in these processes (as described for other tissues) [2].

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