EFFECT OF ORGANOSILICON COMPOUNDS ON PROTEIN BIOSYNTHESIS IN GRANULATION AND FIBROUS TISSUE

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The effect of propoxysilatrane (POS) on biosynthesis of the principal biopolymers of connective tissue was studied. Administration of POS as 0.5 and 2.0% ointments in lanolin-petrolatum base cause stimulation of cell proliferation in granulation and fibrous tissues developing in open skin defects in albino rats. Stimulation of cell proliferation in these animals was shown to be accompanied by increased biosynthesis of collagen and noncollagen proteins. In concentrations of 10^{-3}-10^{-4} M, POS caused intensification of collagen biosynthesis (the formation of peptide-bound nondialyzable hydroxyproline-^{14}C) in vitro in chick embryonic cartilage tissue. The silatranes are thus biologically active substances with a regulatory influence on the course of repair and proliferation in connective tissue.

KEY WORDS: silatranes; connective tissue; protein synthesis; collagen.

The organosilicon compounds known as silatranes have a marked effect on the course of repair and proliferation in connective tissue [1,4]. Connective tissue is known to contain silicon, which is closely bound with its biopolymers [9-11]. A deficiency of silicon in the diet of experimental animals leads to definite disturbances of biosynthesis and function of the biopolymers of connective tissue [7]. It has been shown recently that silatranes have a stimulating effect on the biosynthesis of collagen, one of the principal polymers of connective tissue, in the cartilage tissue of chick embryos [5].

Considering the stimulating effect of silatranes on the course of repair processes and on biosynthesis of connective-tissue polymers, it was decided to study the biochemical characteristics of the effect of one of these compounds on the development of granulation and fibrous tissues.

EXPERIMENTAL METHOD

The compound chosen for study was propoxysilatrane (POS). Experiments were carried out on male and Wistar albino rats weighing 200 g. POS was applied as an ointment in lanolin-petrolatum base (1:1). The concentration of POS in the ointment was 0.5 and 2.0%.

To obtain granulation and fibrous tissue, a circular incision was made in the skin of the dorsal region of the animal in the anterior part of the body, in which a transparent plastic ring was implanted [6]. For 7 days the developing granulation and fibrous tissue was treated with POS ointment. Two groups of animals served as the control: The rats of group 1 received no applications whatever, the animals of group 2 were treated daily with the ointment base consisting of lanolin and petrolatum (1:1). On the 7th day after implantation of the rings, the animals were given a subcutaneous injection of proline-^{14}C in a dose of 10 μCi/100 g body weight. The animals were killed 24 h later and their granulation and fibrous tissue was investigated.

The tissue for study was cut into small pieces with scissors and homogenized in a glass homogenizer with Teflon pestle in 3 ml of 0.5 M CH_3COOH, and made up with the same solution to a volume of 5 ml. Samples of 2 ml of homogenate were treated with 2 ml concentrated HCl and hydrolyzed in glass ampuls at 105°C for 24 h. The concentration of hydroxyproline [12] and radioactivity of hydroxyproline-^{14}C [8] were determined in the digest.

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TABLE 1. Mass of Newly Formed Granulation and Fibrous Tissue

Expt. No.	Group	Mass, g
1	Control	0,81 (0,67—0,96)
2	Lanolin-petrolatum	0,95 (0,78—1,29)
	P_1	—
3	0,5% POS	1,14 (1,10—1,26)
	P_1	0,01
	P_2	0,01
4	2,0% POS	1,03 (0,77—1,20)
	P_1	—
	P_2	—

TABLE 2. Concentration of DNA, RNA, and Hydroxyproline (in g/100 g wet weight of tissue) in Granulation and Fibrous Tissue

Expt. No.	Group	DNA	RNA	Hydroxyproline
1	Control	0,56 (0,33—0,64)	0,46 (0,35—0,51)	0,14 (0,11—0,23)
2	Lanolin-petrolatum	0,50 (0,42—0,62)	0,42 (0,30—0,52)	0,11 (0,075—0,18)
	P_1	—	—	0,05
3	0,5% POS	0,78 (0,55—1,30)	0,53 (0,41—0,55)	0,12 (0,09—0,20)
	P_1	0,001	—	—
	P_2	0,001	—	—
4	2,0% POS	0,76 (0,64—0,85)	0,41 (0,31—0,44)	0,15 (0,12—0,20)
	P_1	0,001	—	—
	P_2	0,001	—	0,05
	P_3	—	—	—

TABLE 3. Biosynthesis of Noncollagen Proteins and Collagen in Granulation and Fibrous Tissue

Expt. No.	Group	Incorporation of hydroxyproline-^{14}C into noncollagen protein		
		cpm/g tissue · 10^{-3}	cpm/mg RNA · 10^{-3}	cpm/μ mole hydroxyproline
1	Control	78,0 (53,0—117,0)	20,8 (11,8—29,9)	798 (575—1260)
2	Lanolin-petroleum	116,3 (101,6—123,4)	21,4 (16,3—29,0)	878 (595—1191)
	P_1	0,001	—	—
3	0,5% POS	132,0 (108,0—217,0)	21,6 (22,6—31,1)	1300 (1060—1490)
	P_1	0,001	0,05	0,001
	P_2	0,05	0,05	0,001
4	2,0% POS	108,5 (72,2—118,8)	25,3 (18,7—26,8)	750 (451—821)
	P_1	0,001	—	—
	P_2	—	—	—
	P_3	0,005	—	0,001

Samples of 2 ml of homogenate were treated with 4 ml 7.5% TCA and hydrolyzed at 100°C for 30 min, after which the concentrations of DNA [3] and RNA in the digest were determined.

To determine radioactivity in the fraction of noncollagen proteins, 0.1 ml homogenate was treated with 3 ml 5% TCA and the samples were heated on a water bath to 90°C for 30 min. The precipitates were then applied to membrane filters (Synpor, No. 6, Czechoslovakia) and radiactivity counted in ZhS-107 scintillation fluid in a liquid scintillation counter (Intertechnique SL-30).

Collagen biosynthesis in chick embryonic cartilage tissue was studied by the method described previously [5].

The results were subjected to statistical analysis by means of the Wilcoxon — Mann — Whitney nonparametric U-criterion [2].

EXPERIMENTAL RESULTS

As Table 1 shows, POS as a 0.5% ointment caused a significant increase in the mass of the granuloma. Under the influence of 0.5 and 2.0% POS ointments an increase also was found in the DNA concentration in granulation and fibrous tissue (Table 2). The increase in the mass of the granuloma, in conjunction with the increased DNA concentration in it, indicates stimulation of cell proliferation under the influence of POS. Besides

TABLE 4. Collagen Biosynthesis in Chick Embryonic Cartilage Tissue under the Influence of POS

Concentration of POS, M	Radioactivity of hydroxyproline-^{14}C, cpm/μmole hydroxyproline	
	control	experiment
10^{-3}	1906	2692
10^{-3}	2124	2421
10^{-3}	2539	2860
10^{-4}	1191	2104
10^{-4}	1600	2100

stimulation of cell proliferation, application of 0.5% POS ointment also was accompanied by intensification of biosynthesis in granulation and fibrous tissues. As Table 3 shows, under the influence of 0.5% POS ointment marked stimulation of collagen biosynthesis took place.

It was shown previously that some silatranes cause stimulation of total protein synthesis and of collagen biosynthesis in vitro in chick embryonic cartilage tissue; moreover, this stimulation is evidently connected with the direct effect of the compound on the protein-synthesizing system of the cell [5]. In the same way POS, in concentrations of 10^{-3}-10^{-4} M, also caused stimulation of collagen biosynthesis in chick embryonic cartilage tissue (Table 4).

In the present experiments stimulation of collagen biosynthesis under the influence of 0.5% POS ointment was accompanied by increased biosynthesis of noncollagen protein (Table 3). These data are evidence that in all probability silatranes not only exert a selective action on collagen biosynthesis, but they also evidently affect the whole process of repair and proliferation through stimulation of cell proliferation, and under these circumstances they also intensify processes of synthesis in cells of granulation and fibrous tissue.

LITERATURE CITED

1. G. A. Grigalinovich, Z. A. Atare, N. V. Milyakova, et al., in: Biologically Active Compounds on Elements of Group IVB. Second All-Union Symposium [in Russian], Irkutsk (1977), p. 136.
2. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria in Medical and Biological Research [in Russian], Leningrad (1973).
3. Z. Dische, in: Nucleic Acids, Chemistry and Biology, E. Chargaff and J. Davidson, eds., Academic Press (1955-1960).
4. L. A. Mansurova, A. G. Platonova, I. G. Kuznetsov, et al., in: Biologically Active Compounds of Elements of Group IVB. First All-Union Symposium [in Russian], Irkutsk (1975), p. 28.
5. B. Z. Simkhovich, T. V. Zamaraeva, G. I. Zelchan, et al., Biokhimiya, 42, 1128 (1977).
6. L. I. Slutskii, in: Biochemistry of Normal and Pathologically Changed Connective Tissue [in Russian], Leningrad (1969), p. 180.
7. E. Carlisle, J. Nutr., 106, 478 (1976).
8. K. Juva and D. J. Prockop, J. Biol. Chem., 241, 4419 (1966).
9. K. Schwarz, Proc. Natl. Acad. Sci. USA, 70, 1608 (1973).
10. K. Schwarz and S. C. Chen, Fed. Proc., 33, 704 (1974).
11. K. Schwarz, Lancet, 1, 454 (1977).
12. H. Stegemann, Hoppe-Seyler's Z. Physiol. Chem., 311, 41 (1958).