EFFECT OF ERYTHROPOIETIC SERUM ON DNA SYNTHESIS IN THE SKIN AND SPLEEN OF MICE DURING CHEMICAL CARCINOGENESIS

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Rat erythropoietic serum containing 0.5 unit erythropoietin (standard B) per ml doubled the intensity of DNA synthesis in the skin of mice treated with 0.04 ml of 0.5% solution of 20-methylcholanthrene in benzene. It had no appreciable effect on normal skin. An increase in the intensity of DNA synthesis in the spleen was observed following administration of this serum whether the carcinogen was applied or not.

Erythropoietin, as a nonspecific growth factor, accelerates the induction of skin tumors [3, 4]. As a specific stimulator of erythroid proliferation, erythropoietin is also known to act on DNA-dependent RNA synthesis [2, 6-9].

In the present investigation the action of erythropoietin was studied on DNA synthesis in the skin of mice. DNA synthesis in the spleen, as an organ rich in cells of the reticulo-endothelial system, and which in mice also plays the role of an active organ of hematopoiesis, not less important than the bone marrow, was determined at the same time.

EXPERIMENTAL METHOD

The usual method of histoautoradiography with tritiated thymidine (thymidine-H^3) with an activity of 5 Ci/ml [1] was used. The number of labeled cells in 70-80 fields of vision, each 10,000μ in diameter, with a magnification of 20 × 60 of the microscope, was counted in the skin in each specimen. Altogether in each group of the experiment 300 fields of vision were counted. For the analysis of the results the mean number of labeled cells per 10,000 μ length of the specimen was calculated.

In the spleen all the labeled cells were counted among 3000 cells in each specimen under a magnification of 10 × 60 of the microscope. Altogether in each group of the experiment 12,000 cells were counted. In the analysis of the results the mean number of labeled cells per 300 cells was calculated.

In these experiments 72 female mice (CBA × C57BL) weighing 20 g were used. Thymidine-H^3 in a dose of 2 μg/g body weight was injected intraperitoneally in 0.2 ml physiological saline into the mice at 7 and 10 A.M. and 1 P.M., 1 h before the animals were sacrificed. The 0.5% solution of 20-methylcholanthrene in benzene was applied to the skin of the interscapular region in a dose of 0.4 ml.

Each group was subdivided into subgroups A, B, and C depending on whether the thymidine was injected at 7 or 10 A.M. or at 1 P. M. The mice of group 1 received an intraperitoneal injection of 1 ml rat erythropoietic serum (EPS) containing 0.5 unit erythropoietin (standard B) [3-5] on the day before application of the carcinogen and during its application (at 11 A. M.); thymidine was injected for the first time next day at 7 A.M. The mice of group 2 received normal serum instead of EPS and acted as the control to group 1. The mice of group 3 were kept under similar conditions, the only difference being that they received physiological saline in the same volume as serum. The mice of group 4 received EPS under the same conditions as those of group 1, but instead of the carcinogen, 0.04 ml benzene was applied to the skin. The mice of

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TABLE 1. Effect of EPS on DNA Synthesis in the Skin and Spleen of Mice (M ± m)

Group No.	Experimental conditions	Subgroup	Time of injection of thymidine	Number of mice	Number of labeled cells in skin	Mean of labeled cells in skin of mice of subgroups A, B, C	P	Number of labeled cells in spleen	Mean no. labeled cells in spleen of mice of subgroups A, B, C	P
1	Injection of EPS 24 h before and during application of carcinogen	A	7 AM	4	2,70±0,43	2,24±0,39	<0,05	15,0±0,82	13,5±0,82	<0,001
		B	10 AM	4	2,13±0,34			11,8±1,03		
		C	1 PM	4	1,90±0,42			13,8±0,63		
2	Injection of normal serum under the same conditions	A	7 AM	4	0,70±0,18	1,09±0,31		5,5±0,36	6,9±0,75	
		B	10 AM	4	1,52±0,48			8,8±0,72		
		C	1 PM	4	1,07±0,27			6,5±1,17		
3	Injection of physiological saline under the same conditions	A	7 AM	4	0,82±0,24	1,27±0,24		4,35±0,30	6,8±1,6	
		B	10 AM	4	1,26±0,26			9,4±2,14		
		C	1 PM	4	1,74±0,22			6,6±2,33		
4	Injection of EPS 24 h before and during application of benzene	A	7 AM	4	1,13±0,29	1,28±0,29		13,5±2,34	13,7±1,59	<0,01
		B	10 AM	4	1,36±0,26			13,8±1,29		
		C	1 PM	4	1,35±0,31			13,8±1,13		
5	Injection of normal serum under the same conditions	A	7 AM	4	1,10±0,35	1,39±0,39		9,1±1,16	7,3±0,93	
		B	10 AM	4	1,70±0,41			5,1±0,84		
		C	1 PM	4	1,38±0,42			7,8±0,80		
6	Injection of physiological saline under the same conditions	A	7 AM	4	1,34±0,58	1,27±0,36		4,8±0,62	4,7±0,58	
		B	10 AM	4	1,44±0,30			3,8±0,55		
		C	1 PM	4	1,02±0,21			5,4±0,58		

group 5 acted as the control to the preceding group and received normal serum. The conditions for the mice in group 6 were the same, but these mice were injected with physiological saline instead of serum.

EXPERIMENTAL RESULTS

The results are given in Table 1. EPS had no effect on DNA synthesis in normal skin (comparison of the animals of group 4 with those of group 5, receiving normal serum). However, in group 1 after application of the carcinogen EPS stimulated DNA synthesis by comparison with the control group 2. For the sake of clarity, the mean values of the results for subgroups A, B, and C were calculated. For instance, whereas in group 1 there were 2.24 labeled cells, for group 2 there were 1.09, i.e., EPS stimulated DNA synthesis in the skin treated with carcinogen up to twice the control level ($P < 0.05$).

In the spleen an equivalent increase in the intensity of DNA synthesis in the whole complement of cells was observed in animals receiving EPS, whether the carcinogen was applied or not, i.e., in this case the erythropoietic serum exhibited its growth property irrespective of the action of the carcinogen.

Administration of EPS thus differed in its effect on normal tissue and on tissue subjected to a single application of the carcinogen. A definite increase in the intensity of DNA synthesis was observed in the skin of the mice after application of the carcinogen, i.e., the carcinogen in this case behaved as a "developer" revealing the nonspecific growth properties of the erythropoietin.

These results suggest that proliferative processes are intensified in tissue treated with the carcinogen and erythropoietic serum. This must evidently be linked with the stimulation of carcinogenesis observed by the writer in the early stages after administration of this serum.

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