

EFFECT OF ACTINOMYCIN D AND ITS COMBINATIONS WITH STEROID HORMONES ON THE BLOOD CLOTTING SYSTEM

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Actinomycin D reduces the clotting power of rabbit blood and the activity of blood coagulation factors II, V, VII, and XIII. Hydrocortisone, prednisone, prednisolone, 6-methylprednisolone, testosterone propionate, and norandrostenolone phenylpropionate all increase the clotting power of the blood, whereas estradiol and desoxycorticosterone have the opposite action. During the combined administration of actinomycin D with glucocorticoids, androgen, and anabolic steroid antagonism between the preparations was found in their action on blood coagulation.

Actinomycin D is a specific inhibitor of RNA synthesis in cells as a result of interaction between this antibiotic and the guanine of DNA. The creation of an experimental model of disturbance of the transcription of genetic information thus enables actinomycin D to be used for the analysis of the primary pharmacological reaction to the administration of biologically active substances [4, 6, 13]. The effect of actinomycin D on the blood clotting system has received inadequate study although it is a problem of both theoretical and practical interest.

The combined administration of actinomycin D with steroid hormones with a marked action on the blood clotting system [5, 7] can reveal the action of the hormones on blood clotting when RNA synthesis is inhibited by the antibiotics.

The object of the present investigation was to examine the mechanism of action of actinomycin D when given alone and in combination with steroid hormones, on the blood clotting system.

EXPERIMENTAL METHOD

Experiments were carried out on 156 rabbits weighing 2000-2500 g, divided into 3 groups. The rabbits of group 1 received actinomycin D in a dose of 50 μ g/kg body weight for 5 days, those of group 2 received hydrocortisone, prednisone, prednisolone, 6-methylprednisolone, desoxycorticosterone (DOCA), testosterone propionate, estradiol, and norandrostenolone phenylpropionate (nerobolil) by intravenous injection daily for 5 days in doses of 1 mg/kg body weight (the duration of the experiment was the same). The rabbits of group 3 received actinomycin D in the doses specified above 1 h before administration of the steroid.

The experimental results were analyzed by statistical methods [1]. In a separate series of experiments the effect of the hormones and actinomycin D in different concentrations on blood clotting was studied in vitro.

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TABLE 1. Effect of Actinomycin D and Steroid Hormones, Alone or in Combination, Administered to Rabbits for 5 Days on Activity of Blood Coagulation Factors

Substance	Dose of hormone (mg/kg)	Dose of actinomycin D (μ g/kg)	No. of animals	Activity of blood clotting factors (in %)		
				II	V	VII
Control	—	—	78	100,7 \pm 3,7	100,5 \pm 3,6	100,7 \pm 4,1
Actinomycin D	—	50	12	38,4 \pm 2,1	54,7 \pm 3,8	32,5 \pm 1,7
Hydrocortisone	1	—	12	141,6 \pm 12,8*	127,5 \pm 10,7*	162,8 \pm 14,3*
	1	50	8	45,2 \pm 4,6	74,8 \pm 4,5	44,2 \pm 2,5
Prednisone	1	—	12	132,1 \pm 15,1*	124,8 \pm 11,4*	150,2 \pm 12,9*
	1	50	8	43,2 \pm 6,8	69,1 \pm 5,4	40,2 \pm 3,7
Prednisolone	1	—	12	129,9 \pm 11,5*	120,2 \pm 11,4*	145,8 \pm 15,8*
	1	50	8	41,3 \pm 2,6	62,5 \pm 8,4	39,7 \pm 2,1
6-Methylprednisolone	1	—	8	125,8 \pm 14,3*	114,8 \pm 18,7	131,4 \pm 11,5*
	1	50	8	48,6 \pm 6,4	59,7 \pm 2,9	46,1 \pm 5,3
Desoxycorticosterone	1	—	8	91,8 \pm 14,1	82,5 \pm 7,2*	78,7 \pm 7,1*
	1	50	8	40,5 \pm 2,5	46,2 \pm 2,8	34,5 \pm 3,8
Estradiol	1	—	8	54,8 \pm 8,1*	70,1 \pm 8,4*	70,4 \pm 9,5*
	1	50	8	41,3 \pm 4,5	54,2 \pm 5,2	49,2 \pm 4,3
Testosterone propionate	1	—	10	124,8 \pm 13,7*	112,5 \pm 15,4	112,5 \pm 15,4
	1	50	6	53,7 \pm 6,9	74,8 \pm 9,4	42,6 \pm 3,8
Noandrosthenolone phenylpropionate	1	—	10	118,4 \pm 24,6	108,5 \pm 12,3	110,7 \pm 12,1
	1	50	6	48,3 \pm 2,6	65,4 \pm 7,3	37,5 \pm 2,4

*Results differ significantly from control ($P < 0.05$).

To assess the effect of actinomycin D and the hormones on the kinetic parameters of blood coagulation, the thromboelastograms were recorded on a "Hellige" apparatus and also on Soviet thromboelastographs of the "Tromb-1" type.

The functional state of the blood clotting system was assessed from the results of measurement of thromboplastic activity by Quick's method [16] and determination of the activity of factors II, V, and VII [11, 12, 15]. Activity of fibrin-stabilizing factor XIII was determined by the method of Sigg and Duckert [17] in Bronshtein's modification [2] with a type SF-4a spectrophotometer [3].

Blood samples were taken for the tests 2, 6, 12, and 24 h after the single injection and also on the 5th day of administration of the hormones or of actinomycin D.

EXPERIMENTAL RESULTS

The experiments in vitro showed that actinomycin D, in concentrations of between 0.1 and 10 μ g/ml, has no effect on the kinetics of blood coagulation when investigated by thromboelastography, nor does it modify thromboplastic activity.

Intravenous injection of actinomycin D into the rabbits in a dose of 50 μ g/kg body weight produced no significant changes in the hemocoagulation potential in thromboelastograms recorded after 2, 6, 12, and 24 h. By the 5th day of administration of the antibiotic in this same dose, the thromboelastograms showed prolongation of the reaction time r from 456.9 \pm 42.5 to 783.1 \pm 39.6 ($P < 0.001$), in the time of commencement of clot formation K from 143.2 \pm 16.5 to 221.4 \pm 6.7 sec ($P < 0.001$), and a decrease in the maximum amplitude of the curves. The activity of the prothrombin complex at this time was reduced from 100.8 \pm 2.0 to 48.7 \pm 2.9%. In addition, activity of blood clotting factors II (prothrombin), V (AC-globulin), and VII (proconvertin) was reduced (Table 1). Activity of factor XIII also was reduced during administration of actinomycin D. These results are in agreement with those obtained by other workers [9, 10, 14].

The experiments of group 2 showed that the steroid hormones investigated had no effect in doses of between 1 and 1000 μ g/ml on blood clotting in vitro.

A single injection of hydrocortisone, prednisone, prednisolone, 6-methylprednisolone, testosterone propionate, and norandrostenolone phenylpropionate into the rabbits increased the clotting power of the blood on the 5th day. This was shown by increases in activity of factors II, V, and VII (Table 1), and also of the fibrin-stabilizing factor.

The combined action of actinomycin D and the above-mentioned hormones over a period of 5 days significantly reduced the hypercoagulatory effect. The thromboelastograms of these animals reflected a state of hypocoagulation although, admittedly, it was not so severe as during administration of the antibiotic alone.

The results suggest that these hormones are evidently inducers of the blood clotting factors studied, and that actinomycin D inhibits their activity. It is a noteworthy fact that the steroid hormones possess an analogous action on the tissue and blood factors of blood coagulation. The writers have previously shown that if actinomycin D is given to rats, the coagulatory activity of the hepatic microsomes is reduced, while the activity of some blood clotting factors is also decreased [8].

Unlike the steroid hormones mentioned above, DOCA and estradiol, in the doses used, depressed the clotting power of the blood on the 5th day of their administration. This was confirmed by lengthening of the r and K intervals of the thromboelastograms and also by the increase in thromboplastin time measured by Quick's method. The activity of blood clotting factors II, V, and VII was lowered by different degrees (Table 1).

The combined administration of DOCA or estradiol and actinomycin D did not result in the summation of their action on blood clotting.

Hence, during administration of steroid hormones combined with actinomycin D, different effects can be obtained on blood coagulation.

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