BIOLOGICAL ACTIVITY OF SPLENIC EXTRACTS CONTAINING DNase I INHIBITOR

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The study of the functional role of natural proteins with high biological activity is very interesting for many branches of biology and medicine. It was shown previously that mouse splenic extracts, of different degrees of purity, increase the survival rate of irradiated mice [5]. Such extracts contained both DNase I and also its natural inhibitor, which belongs to the class of actin proteins [5, 7]. Interaction between serum DNase I and its intracellular inhibitor has been demonstrated in model experiments *in vivo* and also by injection of a synthetic polyanion, giving a marked adjuvant effect, into mice [1, 3]. On this basis it was postualted that the DNase I–inhibitor system takes part in activation of immunocompetent cells [1].

The aim of this investigation was to study the effect of calf splenic extracts, containing DNase I inhibitor, on irradiated animals.

EXPERIMENTAL METHOD

Male $(CBA \cdot C_{57}Bl/6)F_1$ mice weighing 25 g and Syrian hamsters weighing 70-120 g were used. Extracts of different degrees of purity, containing DNase I inhibitor, were obtained by the method in [4] from calf spleens. To obtain the extract, the spleen was freed from membranes, cut into small pieces, then homogenized and centrifuged. The resulting coarse extract was fractionated consecutively on columns with Sephadex G-200 and CM-cellulose (CMC), as described previously [5]. Before chromatography on the CMC columns the samples were subjected to ultrafiltration on semipermeable membranes (from Sartorius Membranfilter, West Germany). Four groups of animals were used: 1) irradiated control, 2) irradiated + coarse extract, 3) irradiated + fraction II from G-200, 4) irradiated + fraction I from CMC. Biological activity of the purified splenic extract was assessed from the survival rate of animals exposed to a single dose of ^{60}Co γ-ray irradiation in doses of 8.15-8.7 Gy, dose rate 0.097-0.106 Gy/min. The test fractions were injected intraperitoneally 60 min after irradiation in a dose of 2.4-10 mg total protein per animal, depending on the level of inhibitor activity in the fractions. Survival of the animals was inspected for 30 days. Activity of DNase I and its inhibitor was determined by the method described previously [2]. Total protein was determined by Lowry's method [8].

EXPERIMENTAL RESULTS

Calf splenic extracts of different degrees of purity were used for the injections: coarse extract, fraction II from G-200, and fraction I from CMC. The results of determination of activity of DNase I and its inhibitor in the splenic extracts and their action on survival of the animals are given in Tables 1 and 2. It will be clear from Table 1 that maximal inhibitor activity was found in fraction II from G-200. Meanwhile activity in fraction I from CMC extract remained at the same level as that of the coarse extract. However, two- or threefold dilution of this fraction with simultaneous increase in the ionic strength of the solutions led to reactivation of the DNase I inhibitor, in agreement with data in the literature [9]. In the course of purification the DNase I activity in the fractions was reduced two- or threefold compared with the coarse extract. It is stated in the literature that the inhibitor forms a specific complex with DNase I in the ratio of 1:1 [6]. Lowering the level of activity of free DNase I in the extracts in the course of purication may shift the equilib-

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TABLE 1. Activity of DNase I and Its Inhibitor in Calf Splenic Extract Fractions

Fraction	Activity/mg protein	
	of inhibitor	of DNase I
Coarse extract	0,100	0,013
Fraction II from G-200	0,200	0,005
Fraction I from CMC	0,080	0,001
The same diluted twofold	0,160	0,001
The same diluted threefold	0,170	0,00

TABLE 2. Survival Rate of Irradiated Animals after Injection of Calf Splenic Extract

Group	Experimental conditions	Mice			Hamsters		
		alive after 30 days	number of animals in experiment	survival rate, %	alive after 30 days	number of animals in experiment	survival rate, %
1	8,7 Gy	6	27	22,2	4	39	10,0
2	8,7 Gy + Coarse extract	5	27	18,5	14	30	47,0*
3	8,7 Gy + Fraction II from G-200	3	18	16,7	4	7	57,0*
4	8,7 Gy + Fraction I from CMC	6	11	54,5*	26	36	72,0†

Legend. Hamsters were irradiated in a dose of 8.15 Gy. P (for survival rate) calculated by $\varkappa$ method for comparison of group 1 with the rest: *P < 0.01, **P < 0.001.

rium DNase I + inhibitor $\rightleftarrows$ DNase I—inhibitor to the left, i.e., it may lead to an increase in activity of the free inhibitor [6].

Investigation of the therapeutic action of splenic extracts containing DNase I inhibitor showed that the survival rate of the irradiated animals increased after injection of the more highly purified fractions obtained on CMC (Table 2). Extracts obtained from calf spleens, as well as extracts obtained from inbred mice, were well tolerated by the animals and gave a therapeutic effect. This effect is evidently not species-specific but depends entirely on the level of inhibitor activity in the samples. Comparison of the irradiated and treated mice and hamsters in group 4 (Table 2) shows a distinct antiradiation therapeutic effect. A therapeutic effect also was observed in hamsters of groups 2 and 3, although survival in mice of these same groups did not differ significantly from that in the control. The explanation is that in order to obtain an antiradiation therapeutic effect in hamsters of groups 2 and 3 the quantity of injected protein in the extracts was increased to 35-40 mg per animal compared with 2.4 mg per mouse in these same groups. It can be tentatively suggested that the effect observed depends on the presence of a DNase I inhibitor which, according to data in the literature, belongs to the group of actin proteins [7]. These proteins play an important role in cell activity: movements, phagocytosis, cytokinesis, and so on. It is stated in the literature that DNase I and its actin protein inhibitor may play a role in association and dissociation of microfilaments [6].

The mechanism of the antiradiation therapeutic action of preparations containing DNase I inhibitor requires further study. The possibility cannot be ruled out that changes in DNase I activity in the serum of the irradiated animals are one factor disturbing intercellular relationships in the body. In this case injection of DNase inhibitor could stablize enzyme homeostasis. Another possibility is that the inhibitor may have a direct action on cells of the hematopoietic organs.

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CHANGES IN BLOOD URIC ACID LEVELS IN PATIENTS WITH RETINITIS PIGMENTOSA AND RATS WITH HEREDITARY DEGENERATION OF THE RETINA

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The writers showed previously that in the early stages of postnatal life of rats with hereditary degeneration of the retina (HDR) (a model of retinitis pigmentosa — RP) in man the 5'-nucleotidase activity of this tissue is modified [4]. The mechanism of development of HDR, according to one hypothesis, is linked with a disturbance of nucleotide metabolism [1], and 5'-nucleotidase is known to be one of the enzymes of purine nucleotide metabolism. The results of biochemical investigation of patients with RP [6] suggest that this disease is accompanied by a raised blood level of uric acid, the end product of purine metabolism in man.

In the view of these data [1, 4, 6], it was decided to attempt to determine whether the blood uric acid level is raised in human subjects with HDR and also to undertake a corresponding investigation on affected rats in order to compare possible changes in metabolism in animal models and in man.

EXPERIMENTAL METHOD

Campbell rats with HDR were used as experimental rats and healthy Wistar rats served as the control group. Animals at different stages of postnatal development were used. The blood uric acid concentration was determined in the patients (27 persons) at the Clinic of the Helmholtz Moscow Research Institute of Eye Diseases. The diagnosis and stage of the disease were determined by the usual clinical tests [2]. Corresponding determinations on healthy subjects (37 persons) served as the control.

TABLE 1. Uric Acid Concentration in Human Serum

Group of subjects	Uric acid concentration, mg%	
	men	women
Healthy	5,8±0,2 (8)	4,6±0,1 (29)
Patients:		
with HDR	8,3±0,6 (14)	7,0±0,7 (13)
with stage II and III of the disease	7,6±0,6 (11)	5,9±0,3 (8)
with stage IV of the disease	9,6±0,5 (3)	8,6±0,4 (5)

Legend. Figures in parenthesis in Table 1 show number of persons, in Tables 2 and 3 number of experiments.

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