SUPPRESSION OF INTRALYSOSOMAL PROTEOLYSIS AGGRAVATES STRUCTURAL DAMAGE AND FUNCTIONAL IMPAIRMENT OF LIVER LYSOSOMES IN RATS WITH TOXIC HEPATITIS

T. A. Korolenko, A. B. Pupyshev,
A. E. Malygin, N. I. Gavrilova,
and N. G. Kurysheva

UDC 616.24-018.15-053.
1-02: 615.277.4

KEY WORDS: lysosomotropic agents; intralysosomal proteolysis; suramin; polyvinylpyrrolidone; toxic hepatitis.

With the aid of lysosomotropic agents, by modifying the properties of lysosomes of target cells in vivo in a certain manner, it is possible to "control" the course of a pathological process [11]. Since lysosomes are involved in the development of cellular damage [3-5], it seems more rational to attempt to stabilize their membranes, and also to "block" their functions so as to prevent or to reduce as far as possible the injurious action of acid hydrolases, liberated intra- or extracellularly.

For this purpose the intralysosomal modifier suramin, which in certain doses, inhibits one of the basis functions of lysosomes, namely digestion of protein [6, 8, 10], was chosen. The compound accumulates mainly in lysosomes of Kupffer cells [8].

The aim of the investigation was to estimate the effect of lowering protein catabolism in the lysosomes on structural and functional properties of the latter during liver damage. For comparison, polyvinylpyrrolidone (PVP), which is inert relative to intralysosomal proteolysis, and which also accumulates largely in lysosomes of the Kupffer cells of the liver [12], was used.

EXPERIMENTAL METHOD

Experiments were carried out on 84 male Wistar rats weighing 170-220 g. Suramin (generously presented by P. D. Hart, Great Britain) and PVP (mol. wt. 24 kilodaltons, from Ferak, West Berlin) were injected simultaneously with CCl_4 in doses indicated previously [2, 3, 6]. The animals were killed 24 and 48 h after CCl_4 poisoning, i.e., in the period of predominance of liver damage.

The preparative and analytical procedures were described in [2, 3, 6]. Serum alanine aminotransferase (AlAT) activity of the rats was determined by the method in [7].

EXPERIMENTAL RESULTS

The development of acute toxic hepatitis in the rats was accompanied by considerable damage to the hepatic parenchyma, as shown by increased AlAT activity (Fig. 1). Similar disturbances of the properties of hepatic lysosomes developed 24 and 48 h after poisoning [3]. To judge from our data, they included labilization of the particles (an increase in free β-galactosidase activity), loss of osmotic properties (reduction of the increase in free β-galactosidase activity and suppression of intralysosomal proteolysis) (Table 1).

Intact rats in which liver function was undisturbed, 24 and 48 h after administration of suramin showed inhibition of intralysosomal proteolysis (Fig. 1; Table 1) and also inhibition of AcP (Fig. 2A) [3, 6]. These parameters served as the basis for the study of accumulation of the compound in rats with hepatitis induced by CCl_4. The fall in the rate of protein catabolism due to suramin is linked with inhibition of thiol-dependent

Central Research Laboratory and Department of Infectious Diseases, Novosibirsk Medical Institute. Novosibirsk Research Institute of Hygiene, Ministry of Health of the RSFSR. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kazhacheev.) Translated by Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 8, pp. 169-172, August, 1985. Original article submitted June 15, 1984.

 0007-4888/85/0008-1040 $09.50

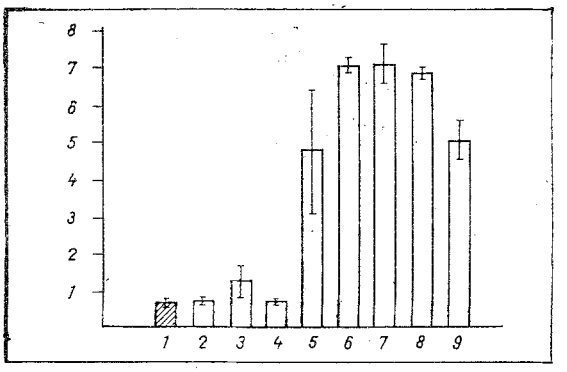

Fig. 1. Effect of suramin and PVP on serum AlAT activity in rats with toxic hepatitis. Abscissa, experimental conditions; ordinate, enzyme activity (in μmoles pyruvic acid/ml/h). 1) Intact rats, 2) suramin (24 h), 3) suramin (48 h), 4) PVP (48 h), 5) CCl_4 (24 h), 6) CCl_4 (48 h), 7) suramin + CCl_4 (24 h), 8) suramin + CCl_4 (48 h), 9) PVP + CCl_4 (48 h).

TABLE 1. Uptake of Labeled Bovine Serum Albumin (^{14}C-BSA) by the Liver and Rate of Intralysosomal Proteolysis (24 h) after Administration of Suramin and CCl_4 to Rats (M±m)

Parameter	Experimental conditions			
	intact rats	suramin	CCl_4	suramin + CCl_4
^{14}C-BSA content, % of injected dose	17,7±1,6	29,5±1,2*	54,9±5,1*	35,3±4,7*
Digestion of ^{14}C-BSA in lysosomal fraction, % of total radioactivity	9,6±1,4	5,7±1,2*	6,0±2,8	5,2±1,4*
Digestion of ^{14}C-BSA in heterolysosomes sedimented with nuclear fraction, % of total radioactivity	11,6±2,3	7,7±1,5	3,8±0,9*	8,8±1,3
^{14}C-BSA acid-insoluble radioactivity, % of control	100,0	151,6	269,5	148,1

Legend. *P < 0.05.

cathepsin B_1 and with a disturbance of lysosomal fusion [1, 6, 8]. Changes in cathepsin D activity in intact rats were not observed under the influence of suramin (Fig. 2b), although data on this question are contradictory [6, 8].

A greater increase in AlAT activity was observed 24 h after injection of suramin and CCl_4 than in "pure" hepatitis, evidence of the more rapid development of the lesion in the hepatocytes. Significant disturbance of this parameter was found in animals of this group after 48 h also (Fig. 1). Under the combined influence of suramin and CCl_4, incidentally, the most intensive labilization of lysosomes corresponded to marked disturbances of the liver function test (Fig. 1).

After injection of suramin and CCl_4 the effect of suramin could be clearly discerned as inhibition of AcP (Fig. 2A) and a decrease in the digestive power of the lysosomes (Table 1). Inhibition of protein catabolism in the lysosomes also took place under the influence of CCl_4, evidently due to a disturbance of the structural integrity of the particles. The increase in acid-insoluble radioactivity in the liver during both separate and combined injection of suramin and CCl_4 reflects a fall in the rate of degradation and, probably, to a lesser degree, increased uptake or protein. Inhibition of intralysosomal proteolysis by suramin evidently leads to a significant disturbance of liver lysosomal function, especially in the Kupffer cells, and this intensifies the process of injury in the organ.

To compare the action of suramin, inhibiting protein catabolism, the lysosomotropic agent PVP, which is inert with respect to proteolysis [12] and also accumulates mainly in the Kupffer cells of the liver [2, 11, 12], was used. Injection of PVP into intact animals was accompanied by an increase in specific AcP activity in liver homogenate (Fig. 2A). The use of PVP together with CCl_4 depressed AlAT activity (Fig. 1) and, at the same time, prevented the development of labilization of lysosomes observed in "pure" hepatitis (Fig. 3A). This was evidence of the beneficial action of PVP in hepatitis induced by CCl_4.

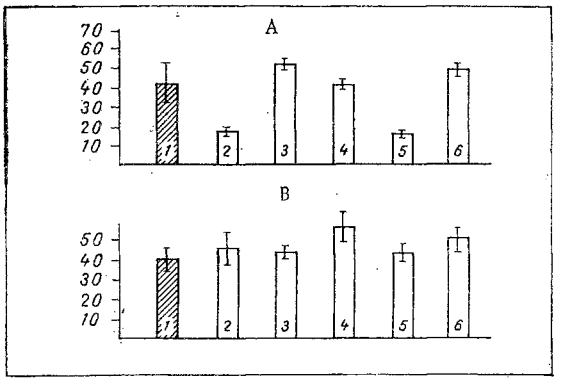

Fig. 2

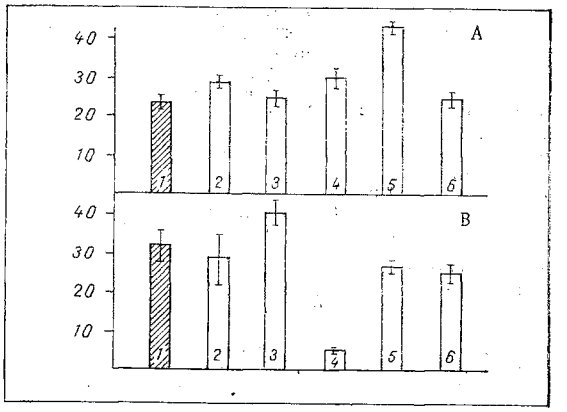

Fig. 3

Fig. 2. Specific activity of acid hydrolases in liver homogenate from rats with toxic hepatitis (48 h) treated with suramin and PVP. Abscissa, experimental conditions; ordinate, specific enzyme activity. 1) Intact rats, 2) suramin, 3) PVP, 4) CCl_4, 5) suramin + CCl_4, 6) PVP + CCl_4. A) AcP) (in μmoles P_i/min/g protein), B) cathepsin D (in μmoles tyrosine/min/mg protein).

Fig. 3. Integrity (stability) and liability to injury of liver lysosomes of rats with toxic hepatitis (48 h) after treatment with suramin and PVP. A) Free β-galactosidase activity in liver homogenate (in % of total activity of enzyme); B) increase in free β-galactosidase activity after treatment of unfractionated liver homogenate in 0.125 M sucrose at 0°C for 30 min (in %). Remainder of legend as to Fig. 2.

These two lysosomotropic compounds, suramin and PVP, thus had opposite actions in toxic hepatitis: the former increased the severity of liver damage and intensified labilization of lysosomes, the latter had a protective effect. To judge from characteristic changes in liver lysosomes induced by suramin (inhibition of AcP, inhibition of intralysosomal proteolysis) and of PVP (increased AcP activity), the damaged organ can ingest both these lysosomotropic compounds. Increased liability of the organelles to osmotic injury during the combined use of these compounds with CCl_4 probably develops on account of loading of the lysosomes (of the Kupffer cells) by the injected compound (Fig. 3B).

When the results are discussed, it is essential to make clear how inhibition of liver lysosomal function affects the course of the pathological process in the organ. The development of injury to the renal parenchyma is known to be essentially determined not only by metabolism of the poison (CCl_4), but also by the state of function of the hepatic Kupffer cells [4, 13–15]. For instance, preliminary injection of colloidal carbon and trypan blue under certain conditions has a protective action in hepatitis induced by CCl_4 [14, 15], the mechanism of the Kupffer cells, where these compounds accumulate, determine their beneficial effect in liver damage [4, 14, 15].

It has been suggested that lysosomes of the hepatic Kupffer cells play an essential role in the course of both injury and repair processes in the organ [4]. However, it is not clear how this effect takes place. The results of the present investigation show that inhibition of proteolysis by suramin intensifies structural and functional disturbances of the lysosomes in the liver and increases damage to the organ. A direct link evidently exists between these phenomena, and this is confirmed by the fact that PVP, which is inert in relation to intralysosomal proteolysis, has the opposite effect. The lysosomotropic properties of suramin enable the worsening which is observed to be connected with disturbance of lysosomal function, especially in the Kupffer cells of the liver, where the compound accumulates. However, the role of inhibition of nonlysosomal enzymes by suramin cannot be completely ruled out [8, 9]. Functioning of the lysosomes of the Kupffer cells is evidently essential for protection of the organ under pathological conditions.

The results suggest that it is risky to use drugs which inhibit intralysosomal proteolysis in the treatment of patients with acute hepatitis.

LITERATURE CITED

1. G. Ya. Vidershain, Biochemical Basis of Glucosidoses [in Russian], Moscow (1980).
2. N. I. Gavrilova, A. B. Pupyshev, and T. A. Korolenko, Byull. Éksp. Biol. Med., No. 7, 58 (1982).
3. T. A. Korolenko, Byull. Éksp. Biol. Med., No. 3, 234 (1979).

4. D. N. Mayanskii, The Kupffer Cell and System of Mononuclear Phagocytes [in Russian], Novosibirsk (1981).
5. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
6. A. P. Pupyshev, A. E. Malygin, and T. A. Korolenko, Biokhimiya, No. 7, 1167 (1981).
7. E. Szczeklin, Fundamentals of Enzymology [in Russian], Warsaw (1978).
8. C. H. C. M. Buys, J. M. W. Bouma, M. Gruber, et al., Naunyn-Schmiederberg's Arch. Pharmakol., 304, 183 (1978).
9. G. Constantopoulos, S. Rees, B. G. Cragg, et al., Biochem. Biophys. Res. Commun., 101, 1345 (1981).
10. M. Davies, J. B. Lloyd, and F. Beck, Biochem. J., 121, 21 (1971).
11. C. de Duve, T. de Barsy, B. Poole, et al., Biochem. Pharmacol., 23, 2495 (1974).
12. J. Munniksma, T. Noteborn, S. Stientstra, et al., Biochem. J., 192, 613 (1980).
13. G. Paumgartner, J. Longueville, and C. M. Leevy, Exp. Mol. Pathol., 9, 161 (1968).
14. M. Petrelli and R. Stenger, Exp. Mol. Pathol., 10, 115 (1969).
15. R. J. Stenger, M. Petrelli, A. Segel, et al., Am. J. Pathol., 57, 689 (1969).

EFFECT OF HYPOKINESIA AND MUSCULAR TRAINING ON STROKE VOLUME CONTROL PATTERNS IN RATS

R. A. Abzalov and R. R. Sitdikova

UDC 612.173.1.018: 577.175.52]-06:] 612.766.2+ 612.745

KEY WORDS: stroke volume of the heart; hypokinesia. muscular training.

Data in the literature on the effect of muscular training on the cardiac output of the developing organism and contradictory [5, 7, 11] and do not reveal their controlling mechanisms. The character of control of the cardiac output in the growing organism during hypokinesia likewise has not been studied.

It was accordingly decided to investigate the formation and modification of the tonic influence of the sympathetic and parasympathetic divisions of the autonomic nervous system on the stroke volume (SV) of the heart in young rats kept for a long time under conditions of hypokinesia or subjected to muscular training.

EXPERIMENTAL METHOD

Noninbred rats aged 21 days were divided into several groups. The rats of group 2 were kept for 49 days under constrained conditions of hypokinesia [2]. Animals of group 3 were kept in ordinary training cages, with 6 to 8 rats in each cage (control). Animals of group 4 were adapted for 49 days to gradually increasing muscular exercise by swimming (trained rats) [1]. Acute experiments were carried out under urethane anesthesia (600 mg/kg). SV was determined by tetrapolar rheography [10] with the RPG-204 apparatus, made by the No. 1 Experimental Production Workshops, Academy of Medical Sciences of the USSR. Specific resistance for animals of each group was determined beforehand (data not given). Tone of the sympathetic and parasympathetic nerves was judged from changes in SV after pharmacologic blockade of the corresponding receptors. To block sympathetic nerves the animals were given a subcutaneous injection of a 0.1% solution of propranolol hydrochloride (Isis-Chemie, East Germany) in a dose of 0.8 mg/100 g body weight. Acetylcholine receptors of the heart were blocked by fractional injection of atropine (0.1%) in an average dose of 0.3 mg/100 g, divided into several portions.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. The value of SV in rats of the control group aged 10 weeks was 6.5 times higher than at the age of 3 weeks, i.e., the cardiac output increased with growth of the animal, in agreement with data in the literature [6, 7, 9]. In rats kept under conditions of hypokinesia, SV was

Department of Physiology, Pedagogic Institute, Kazan'. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 8, pp. 172-174, August, 1985. Original article submitted January 18, 1985.

