LITERATURE CITED

1. Yu. V. Burov, G. I. Absava, A. B. Kampov-Polevoi, et al., Farmakol. Toksikol., No. 1, 50 (1981).
2. A. I. Maiskii and N. N. Vedernikova, Usp. Sovrem. Biol., 87, No. 2, 199 (1979).
3. Yu. P. Naidenov, in: Proceedings of a Conference on Biology of Laboratory Animals [in Russian], Moscow (1967), p. 82.
4. I. A. Sytinskii, Biological Bases of the Action of Ethanol on the Central Nervous System [in Russian], Moscow (1980).
5. A. C. Church, J. L. Fuller, and L. Dann, J. Comp. Physiol. Psychol., 93, 242 (1979).
6. R. W. Farmer and L. F. Fabre, Adv. Exp. Med. Biol., 56, 277 (1975).
7. E. M. Hakansson and L. Östberg, Biochem. Genet., 14, 771 (1976).
8. T. K. Li. L. Lumeng, W. J. McBride, et al., Drug Alcohol Depend., 4, 45 (1979).
9. L. Schuster, G. W. Webster, G. Yu, et al., Psychopharmacologia, 42, 249 (1975).
10. A. Svejgaard and L. P. Ryder, Lancet, 2, 547 (1976).
11. B. Tabakoff and R. F. Ritzmann, Drug Alcohol Depend., 4, 87 (1979).

EXPERIMENTAL STUDY OF POLYVINYLPYRROLIDONE BINDING BY LYSOSOMES

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UDC 615.384.015.44:616.36-018.11:576.311.342

KEY WORDS: polyvinylpyrrolidone; rat liver lysosomes; lysosomotropic agents.

Lysosomotropic agents, which include chlorazine, streptomycin, iron compounds, etc., are a group of compounds which, when administered *in vivo*, are selectively accumulated in the subcellular particles known as lysosomes [4, 8]. Lysosomotropic properties of therapeutic compounds, both negative and positive, have been insufficiently studied; moreover, this effect has so far been virtually ignored both by clinicians and by pharmacologists [8].

Compounds of polyvinylpyrrolidone (PVP) are used in medicine [1, 2, 5, 6]. Low-molecular-weight PBP with mol. wt. 12,600 ± 2700, is a component of the Soviet product Gemodez, used for detoxication purposes [2, 6]. A PVP with average molecular weight (28,000-60,000) is a component of antishock fluids as a plasma expander [5, 6]. A characteristic property of PVP compounds is their marked ability to form complexes [6]; the preparations can bind toxins circulating in the blood stream and eliminate them by excretion through the kidneys [5, 6]. However, the mechanism of action of PVP has not been fully explained. It can be tentatively suggested that binding of PVP with the toxin also takes place in cells of the reticuloendothelial system, the intensive functioning of which leads to detoxication. It has been shown that repeated injections of low-molecular-weight PVP leads to an increase in specific activity of the lysosomal enzyme acid phosphatase [10]. However, binding of the compound with liver cells and changes in the physicochemical properties of lysosomes of the liver cells has not been studied. It can be postulated that the detoxication properties of PVP are largely associated with the affinity of preparations for lysosomes.

The aim of the present investigation was to study the structural and functional properties of lysosomes in rat liver cells after administration of PBP with mol. wt. of 24,000 and 30,000.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g (15 control and 15 experimental animals) were used. PVP (mol. wt. 24,000, from Ferak, Berlin) was injected daily, intraperitoneally for 3 days at 25 h intervals, in the form of a 6% solution in 0.9% NaCl, in a dose of 3.3 ml/100 g body weight [10]. The animals were decapitated 48 h after the last injection. Intact rats served

Department of Infectious Diseases, Central Research Laboratory, Novosibirsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 7, pp. 58-60, July, 1982. Original article submitted July 8, 1981.

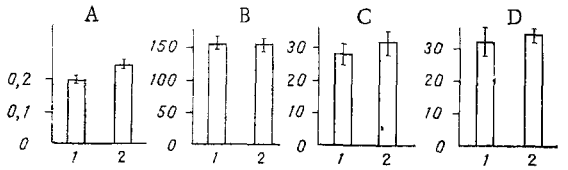

Fig. 1. Changes in specific activity of lysosomal enzymes of rat liver after three injections of PVP (mol. wt. 24,000). Ordinate, specific enzyme activity: A) acid phosphatase (in μmoles Pi/min/g protein); B) acid ribonuclease (O.D.260 nm/min/mg protein); B) cathepsin D (O.D. 280 nm/min/g protein; C) β-galactosidase (μmoles p-nitrophenol/min/g protein). 1) Intact animals; 2) animals receiving three injections of PVP.

TABLE 1. Integrity and Vulnerability of Rat Liver Lysosomes *in vitro* after Three Injections of PVP

Enzyme	Intact rats	Animals receiving three injections of PVP
Acid phosphatase:		
free activity	21,1±0,79	26,3±1,35*
hypotonic treatment	48,9±1,41	59,3±1,56*
Acid ribonuclease:		
free activity	24,9±0,75	28,2±0,75*
hypotonic treatment	48,8±1,91	65,5±1,44*
Cathepsin:		
free activity	28,0±0,84	34,4±1,44*
hypotonic treatment	66,8±1,43	94,9±1,61
β-galactosidase:		
free activity	36,2±2,43	31,8±1,29
hypotonic treatment	78,9±4,12	86,6±5,03

Legend. Vulnerability of lysosomes in hypotonic medium determined after treatment of rat liver homogenate in 0.125 M sucrose at 0°C for 15 min [12]; results expressed as free enzyme activity (in % of total activity). *$P < 0.05$.

as controls. A liver homogenate was prepared by following the recommendations for studying the state of liver lysosomes [4]. The integrity of the lysosomes was estimated from the level of free activity of acid hydrolases in the liver homogenate; the vulnerability of the particles in a hypotonic medium was determined by the method of Neely et al. [12]. The results were expressed as percentages of total activity. Total activity of the lysosomal enzymes (acid phosphatase, acid ribonuclease, cathepsin D, β-galactosidase) in liver homogenate was determined in the presence of 0.1% Triton X-100. The recommendations of Barrett [7] were used when measuring acid hydrolase activity.

To study uptake by the organs and the subcellular distribution in the liver cells, ^{125}I-PVP, from the Radiochemical Centre, Amersham, England, with mol. wt. 30,000-40,000 and with specific radioactivity at the time of sacrifice of the animals of about 15 μCi/mg, was used. The ^{125}I-PVP was injected intravenously in a dose of 1 mg/kg body weight in the form of a 1% solution in 0.9% NaCl, 5 days before sacrifice. For counting γ-radioactivity in the samples, 0.05 ml of the original solution of ^{125}I-PVP was used as the standard. The content of the compound in the supernatant fraction was determined after centrifugation of the liver homogenate at 105,000g for 1 h.

The results were subjected to statistical analysis, using Student's criterion.

EXPERIMENTAL RESULTS

Five days after injection of ^{125}I-PVP into the rats, compared with the kidneys and spleen the liver showed a higher uptake of the compound: The corresponding values were 0.43 ± 0.07, 0.93 ± 0.02, and 7.3 ± 0.34% of the injected dose (data for 10 animals).

These results agree with those of Munniksma et al. [11], who studied uptake of PVP with mol. wt. of 33,000. They show that uptake of the compound by the liver cells takes place on account of liquid pinocytosis, at a slow rate.

PVP taken up by endocytosis in the liver was located (about 90%) in the lysosomes. Evidence of the lysosomal localization of the ingested PVP was given by the following results of these experiments: 90% of the ^{125}I-PVP was bound with the granular fractions and 10% of the total radioactivity of the liver homogenate was bound with the supernatant fraction (105,000g). On treatment of the liver homogenate with 0.1% Triton X-100, the lysosomes were destroyed and 90% of the labeled PVP, like the lysosomal enzyme β-galactosidase, was found in the supernatant. Consequently, solubilization of ^{125}I-PVP takes place in a similar manner to liberation of the lysosomal enzymes. The results indicate the lysosomal localization of the compounds, and also the low degree of binding of ^{125}I-PVP with the lysosomal membranes.

Electron microscopically, heterolysosomes containing electron-translucent material were found more often in the Kupffer cells of the liver than in hepatocytes [3]. Rat liver cell lysosomes are known not to contain enzymes capable of degrading synthetic PVP polymer. Intralysosomal accumulation of PVP is accompanied by secondary changes in the particles, which include an increase in specific acid phosphatase activity (Fig. 1) and changes in the physicochemical properties of the particles [13, 15].

To judge from the increase in free activity of acid phosphatase, acid ribonuclease, and cathepsin D after three injections of PVP, labilization of the liver lysosomes occurred: The vulnerability of the particles in a hypotonic medium was increased (Table 1). Increased sensitivity to the action of hypotonic treatment is considered to reflect volume disturbances of the lysosomes, i. e., an increase in their radius. The changes were detected during a study of enzymes (cathepsin D, acid ribonuclease) whose activity is higher in the Kupffer cells of the liver [9], and also of acid phosphatase, located chiefly in the hepatocytes. Consequently PVP, judging from changes in the lysosomal enzymes of the liver, accumulates both in lysosomes of hepatocytes and in Kupffer cells of the rat liver. The intralysosomal accumulation of the compound induces a series of changes in the properties of the particles, which resemble those occurring after injection of other unmetabolized lysosomotropic preparations such as Triton WR-1339, sucrose, etc. [14].

The results of the present investigation confirm the lysosomal localization of PVP. Since the doses of the compound used in the experiments were sufficiently high it can be concluded that under clinical conditions the lower doses of low- and average-molecular weight PVP will place a smaller load on the lysosomes. However, during repeated injections of Gemodez into patients, the load on the lysosomes may be increased.

It can be postulated that intralysosomal accumulation of PVP in the liver cells, especially the Kupffer cells helps to stimulate their endocytic activity and, in particular, their ability to take up and excrete complexes formed by PVP with toxins. In this case the favorable aspects of the affinity of the compound for lysosomes, thanks to which PVP has a protective, detoxicating action in various diseases in man, are exhibited.

LITERATURE CITED

1. P. S. Vasil'ev, V. V. Suzdaleva, N. M. Gyul'badamova, et al., Probl. Gematol., No. 10, 28 (1967).
2. G. M. Vidavskaya, V. V. Suzdaleva, and N. S. Rozanova, in: Abstracts of Proceedings of the 43rd Plenum of the Scientific Council of the Central Institute of Hematology and Blood Transfusion [in Russian], Vol. 2, Moscow (1967), pp. 266-267.
3. V. N. Gavrilin and N. I. Gavrilova, in: Structure and Functions of Lysosomes [in Russian], Novosibirsk (1980), pp. 47-48.
4. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
5. I. M. Rabinovich, The Use of Polymers in Medicine [in Russian], Leningrad (1972.
6. F. P. Sidel'kovskaya, Chemistry of N-Vinylpyrrolidone and Its Polymers [in Russian], Moscow.
7. A. J. Barrett, in: Lysosomes. A Laboratory Handbook, (J. T. Dingle, ed.), Amsterdam (1972), pp. 110-126.
8. C. De Duve, T. De Barsy, B. Poole, et al., Biochem. Pharmacol., 23, 2495 (1974).
9. D. L. Knook and E. C. Sleyster, Mech. Ageing Dev., 5, 389 (1976).
10. A. Mijer and R. Willighagen, Biochem. Pharmacol., 12, 973 (1963).
11. J. Munniksma, M. Noteborn, T. Kooistra, et al., Biochem. J., 192, 613 (1980).
12. A. N. Neely, P. B. Nelson, and G. E. Mortimore, Biochim. Biophys. Acta, 338, 458 (1974).
13. L. Ose, T. Ose, R. Reinersten, et al., Exp. Cell Res., 126, 109 (1980).
14. K. E. Williams, E. M. Kidston, F. Beck, et al., J. Cell Biol., 64, 113 (1975).