PHARMACOKINETICS OF A WATER-SOLUBLE ANTI-OXIDANT OF 3-HYDROXYPYRIDINE TYPE

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An intensive search for physiologically active substances among derivatives of 3-hydroxypyridine (3-HP), which are water-soluble antioxidants of biogenic type and structural analogs of compounds of the vitamin B_6 group, and the study of the mechanism of their action are currently in progress. It has been shown that 3-HP derivatives possess a broad spectrum of psychopharmacologic action which, in particular, embraces anxiolytic, antistressor, antihypoxic, and anticonvulsant effects [2, 4, 5]. Meanwhile the mechanisms of the psychotropic action of compounds of this type have not yet been explained, so that there is a need for molecular-biological and pharmacokinetic investigations of 3-HP derivatives.

The aim of the present investigation was accordingly to study the pharmacokinetics of a member of this group of antioxidants, namely a water-soluble salt of 2-ethyl-6-methyl-hydroxypyridine (EM-3-HP).

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 160-180 g. EM-3-HP was injected intraperitoneally in a dose of 150 mg/kg. Its concentration in the blood plasma, liver, and brain was determined 15, 30, and 45 min and 1, 2, 3, 4, 6, 8, and 24 h after injection by high performance liquid chromatography. To study binding of EP-3-HP with cell membranes, 2, vols. % of Triton X-100 was added to the liver and brain homogenates from the animals before extraction. By centrifugation in a sucrose gradient the binding capacity of membranes of the endoplasmic reticulum for EM-3-HP was studied [3].

EM-3-HP was extracted by the addition of 0.1 ml of 0.05 M Tris-HCl (pH 8.0) and 0.2 ml of a 20% solution of SDS to 2 ml of plasma, followed by thorough mixing for 5 min. The mixture was then treated with an equal volume of benzene, shaken vigorously for 15 min, and then centrifuged at 10,000 rpm for 15 min to separate the aqueous (bottom) and benzene (top) layers. The aqueous layer was carefully withdrawn, and the whole extraction procedure described above was repated with it. Aqueous and benzene layers were pooled and evaporated at 90 and 70°C respectively. The procedure of obtaining extracts of EM-3-HP from the tissues consisted essentially of homogenization of the tissue in 4 volumes of 0.01 M Tris-HCl, pH 7.7 (0°C) in a Counce glass homogenizer followed by sedimentation of large tissue particles by centrifugation at 700 rpm for 15 min. The subsequent extraction procedure was similar to that described above for plasma.

Dry residues obtained by evaporation were dissolved in 1 ml of a 10% aqueous solution of methanol. Next, 25 µl of the solution was introduced into the injector of an "Altex-Tracor 970 A" chromatographic system and analyzed in an "Alltech C-6000" column (4.6 × 250 mm) on a stationary phase of "Spherisorb–CN5S" using a methanol–water system (1:9 v/v) as the eluent. The conditions of chromatography were: rate of flow of eluent 1.0 ml/min, column temperature 25°C. The chromatograms were recorded automatically with respect to absorption at 296 nm. The EM-3-HP fractions were identified on the basis of chromatography of a pure standard and also of the results of UV-spectrophotometric scanning and mass spectrometry. The quantity of EM-3-HP in the chromatographic fractions was determined by normal-

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TABLE 1. Pharmacokinetic Parameters of EM-3-HP in Rats

Tissue	Parameter								
	Ka, h^{-1}	$T_{1/2a}$, h	Ke, h^{-1}	$T_{1/2e}$, h	V, liters	T_{max}, h	C_{max}, ng/ml	Cl_p, liters/h	AUC, ng·h/ml·kg
Liver	1,04	0,66	0,13	5,33	653	2,3	171	84,9	1767
Liver (Triton X-100)	0,81	0,85	0,10	6,97	309	2,9	363	30,7	4887
Brain	0,69	1,01	0,24	2,86	582	2,3	146	141,0	1061
Brain (Triton X-100)	0,35	1,96	0,36	1,92	147	2,8	373	52,8	2840
Plasma	0,74	0,94	0,11	6,38	533	3,0	202	57,8	2594

TABLE 2. Concentration of EM-3-HP (in ng/mg protein) in Different Subcellular Fractions of Rat Liver and Brain after Intraperitoneal Injection in a Dose of 150 mg/kg

Time after injection, h	Membranes of endoplasmic reticulum		Cytosol	
	liver	brain	liver	brain
2	380	307	110	95
24	140	102	32	40
48	80	64	—	—
72	30	50	—	—

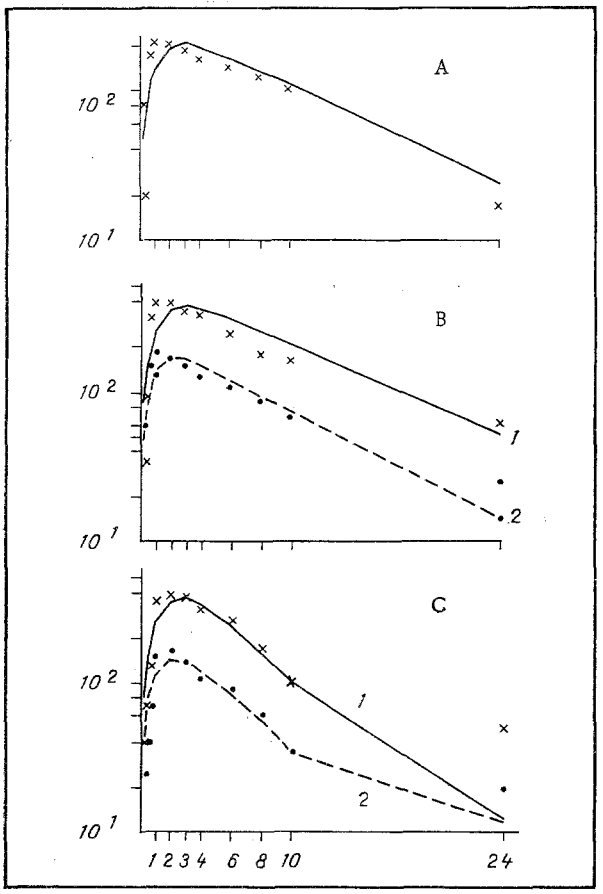

Fig. 1. Kinetic curves of EM-3-HP levels in plasma (A), liver (B), and brain (C) after intraperitoneal injection in a dose of 150 mg/kg. Abscissa, time (in h); ordinate EM-3-HP concentration: A) in ng/ml, B, C) in ng/g. 1) Extraction from homogenate after addition of Triton X-100, 2) extraction from homogenate without addition of Triton X-100.

ization with a Chromatopac C-R1A integrator. The kinetics of the unchanged preparation was interpreted by the use of a one-chamber model with suction.

The sedative action of EM-3-HP was assayed by recording changes in motor activity by means of an actometer (Ugo Basile, Italy).

EXPERIMENTAL RESULTS

Kinetic curves of EM-3-HP levels in the rats' blood and tissues are shown in Fig. 1, and the calculated parameters of kinetics are given in Table 1. The results showed that EM-3-HP in rats is absorbed fairly rapidly from the peritoneal activity, with a half-absorption time of 0.94 h, and that maximal plasma level are achieved after 3 h. Comparison of the half-elimination times of EM-3-HP from different biological substrates showed that the substance is eliminated most rapidly from the rat brain. Maximal concentrations in the animals' brain and liver, just as in their plasma, were reached after 2-3 h, and amounted to 146, 171, and 202 ng/ml respectively.

After treatment of the liver and brain homogenates with Triton X-100 higher concentrations of EM-3-HP were found at the corresponding times after injection (Fig. 1). In this case the highest concentration in the liver and brain was more than doubled (Table 1). These data indicate that the increase in concentration of EM-3-HP in the animals' organs and slowing of its release were due to binding of the substance with liver and brain cell membranes. When the EM-3-HP clearance was compared in the liver,brain, and plasma of the rats it was found that whereas the unchanged substance was released more rapidly from whole brain homogenate than from the animals' plasma and liver homogenate, if allowance was made for the kinetics of elimination of membrane-bound forms of EM-3-HP, this relationship was altered: the total quantity of the substance was eliminated more rapidly from plasma than from the rats' brain and, in particular, from their liver (Table 1).

The results of the study of the binding capacity of endoplasmic reticulum membranes of the liver and brain for EM-3-HP confirmed data given above (Table 2). For instance, EM-3-HP was found in high concentrations in membranes of the endoplasmic reticulum from the animal organs for a period of 72 h, whereas in the cytosol it was found for only 24 h and in much lower concentrations.

To discover any possible connection between the pharmacokinetic relationship and the pharmacologic activity of EM-3-HP, the pharmacodynamics of this compound was studied with respect to its sedative action. The pharmacologic effect was found to develop gradually: it amounted to 30% 15-30 min after injection, reached a maximum (50-80%) after 1-2 h, remained at a high level for 3-4 h, and then gradually disappeared.

Definite dependence between the pharmacokinetic and pharmacodynamic parameters was thus discovered in the action of EM-3-HP.

Consequently, the pharmacokinetic study of this water-soluble antioxidant indicates that it is a compound with membranes activity. It can be tentatively suggested that this substance, which can be found for a long time in the membranes, is able to modify their physicochemical properties and functional activity and, through its membrane-modulating action, it is thus able to exhibit its pharmacologic action.

The long stay of this antioxidant in the membranes may also be responsible for its membrane-protective action, revealed during protection of erythrocyte membranes against hemolysis [4], and also its membrane-modifying action, leading to changes in the phospholipid composition and functional activity of biological membranes [1].

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