Inhibition of Human Serum Dopamine β-Hydroxylase After the Oral Administration of Fusaric Acid

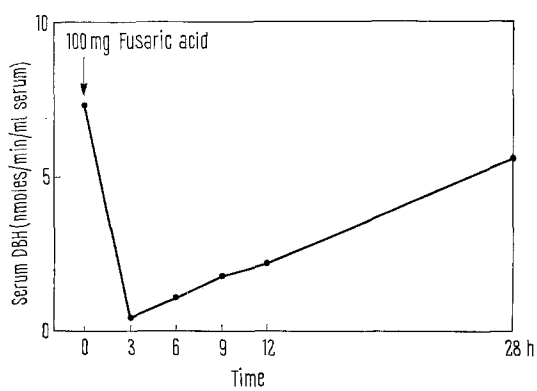
Fusaric acid (5-butylpicolinic acid) was found to be a potent inhibitor of dopamine β-hydroxylase in vitro and a potent hypotensive agent[1,2] in rat, rabbit and dog. A more pronounced hypotensive effect of fusaric acid was observed on spontaneously hypertensive rats, which had been produced by OKAMOTO and AOKI[3], than on normotensive Wistar rats[4]. The compound inhibited dopamine β-hydroxylase in adrenal glands in vivo and reduced the endogenous level of tissue catecholamines[2]. The hypotensive action of fusaric acid in humans was also reported[5].

In order to see the degree of inhibition of dopamine β-hydroxylase in vivo during the oral administration of fusaric acid to humans, serum dopamine β-hydroxylase activity[6,7] has been measured. Human serum dopamine β-hydroxylase activity was measured by the radioassay of WEINSHILBOUM and AXELROD[6]. Healthy male subjects (age range, 30–40 years) took 50–300 mg of the calcium salt of fusaric acid per os. Blood samples were obtained by venopuncture and immediately placed on ice. Serum was removed after centrifuging the blood at 10,000×g for 10 min. Incubation mixture for the enzyme assay (310 µl) contained: 10 µl of serum as enzyme; water, 170 µl; 1 *M* *Tris*-HCl buffer, pH 6.0, 10 µl; 40 m*M* ascorbic acid in the *Tris* buffer, 30 µl; 40 m*M* sodium fumarate, 30 µl; 6 m*M* pargyline, 10 µl; 0.05 m*M* $CuSO_4$, 30 µl; 30 m*M* tyramine, 10 µl; and catalase (5 mg crystals in 2 ml of water), 10 µl. Incubation was carried out at 37°C for 20 min. After the incubation the following reaction mixtures were added: 1 *M* *Tris*-HCl buffer, pH 8.6, 80 µl; 0.1 m*M* EDTA, 200 µl; phenylethanolamine N-methyltransferase partially purified from bovine adrenal medulla by the method of CONNETT and KIRSHNER[8] (0.17 nmoles/min/mg protein, 2.4 mg protein/ml), 10 µl (24 µg): S-adenosyl-methionine-[methyl-C^{14}], 20 µl (0.1 µCi, 1.8 nmoles). The incubation was continued further for 30 min at 37°C. The radioactive N-methyl-octopamine formed was extracted into toluene-isoamyl alcohol (3:2, v/v) and counted. Boiled serum (95°C, 5 min) was used for the blank incubation. Octopamine, 0.8 nmole, was added into a reaction mixture as an internal standard.

Serum dopamine β-hydroxylase activity of 10 normal Japanese subjects (male, 30–40 years of age) was 9.1±0.9 (S.E.) nmoles/min/ml serum or 0.13±0.02 (S.E.) nmoles/min/mg protein. As shown in the Table, serum dopamine β-hydroxylase activity was completely inhibited up to 12 h after the oral administration of 300 mg of the calcium salt of fusaric acid. Recovery of the enzyme activity was noticed 24 h after the administration. After the administration of 100 mg of the calcium salt of fusaric acid, marked inhibition (70–95%) of the serum enzyme activity was observed at 3 h, and the activity gradually recovered as shown in the Figure. 24 h after the administration, the activity recovered to about 90% of the initial level. After the administration of 50 mg of the calcium salt of fusaric acid, about 60% inhibition of the serum enzyme activity was observed at 3 h, and the activity almost completely recovered after 24 h.

The results indicated that fusaric acid is a potent inhibitor of dopamine β-hydroxylase in vivo in humans and that the assay of the serum enzyme activity could be a convenient biopsy for monitoring the degree of enzyme inhibition in vivo in clinical studies.

It was previously shown that the inhibition of the pure dopamine β-hydroxylase by fusaric acid is completely reversed by dialysis[2]. In order to see whether or not the inhibition by fusaric acid is recovered reversibly following the removal of fusaric acid from the serum, the serum preparations 3 h after the administration of fusaric acid, which had no activity, were treated with ammonium sulfate fractionation and then dialyzed against phosphate buffer. However, no activity appeared after the isolation procedure of the enzyme. Thus, the result indicated that



Time course of the inhibition of serum dopamine β-hydroxylase activity after oral administration of fusaric acid.

Inhibition of serum dopamine β-hydroxylase activity after oral administration of fusaric acid

Subject No.	Fusaric acid (Calcium salt) (mg)	Dopamine β-hydroxylase activity (%) Time after administration (h) 0	3	6	9	12	24
1	300	100	2	1	0	0	11
2	300	100	0	0	—	—	48
3	300	100	1	2	—	—	85
4	100	100	5	15	25	29	77
5	100	100	32	—	—	—	91
6	100	100	29	—	—	—	88
7	50	100	43	—	—	—	91
8	50	100	31	—	—	—	97

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the inhibition of serum dopamine β-hydroxylase by fusaric acid in vivo may become irreversible. Another possible explanation may be that fusaric aid inhibits the discharge of the enzyme from the sympathetic nerve endings. This problem remains for further investigation[9].

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Zusammenfassung. Die Dopamin-β-Hydroxylase-Aktivität im menschlichen Serum wurde nach der oralen Zufuhr von Fusarinsäure (50–300 mg) stark gehemmt.

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Non-Membrane-Bound Cytoplasmic Deposits in Krabbe Globoid Leukodystrophy: Further Evidence for a Revised Concept of Lysosomal Storage Diseases

In the past decade, biochemical studies have revealed that the essential defect underlying many of the inherited storage diseases is a genetically determined block in one or more degradative pathways, i.e., a lacking or extremely lowered activity of one or more catabolic enzymes. These are mainly hydrolases with acid pH optimum largely localized within cytoplasmic organelles defined by and comprised in the lysosome concept. It is, therefore, not astonishing that, in various thesaurismoses, the respective storage materials were found to be deposited for the most part inside membrane-bound cytoplasmic bodies or vacuoles of lysosomal nature. Accordingly, the term 'inborn lysosomal storage diseases' was introduced to designate metabolic errors of this kind[1,2]. As to the mechanisms by which the storage substances gain entrance into and can be concentrated within the lysosome system, two pathways have been postulated: cellular autophagy and endocytosis[1,2]. In the case of deficient activity of one or more acid hydrolases normally carried into autophagic and heterophagic vacuoles by primary lysosomes, the natural substrates of these enzymes cannot be cleaved during digestion of the vacuole contents, and thus remain as inert residues within the vacuoles. Repetition of such an incomplete degradative process must necessarily cause an accumulation of indigestible substances inside an increasing number of secondary lysosomes and/or residual bodies[1,2].

Krabbe globoid leukodystrophy (GLD) is in essence a hereditary disorder of galactocerebroside metabolism transmitted most probably as an autosomal recessive trait[3]. We examined electron microscopically a brain biopsy from a child with GLD, and we observed numerous cells containing the multiangular polymorphous inclusions which are said to be characteristic of Krabbe's disease and to consist for the most part of galactocerebrosides[4]. It was remarkable that these deposits were present by no means exclusively within membrane-bound lysosome-like bodies or vacuoles of variable size and appearance. Many inclusions were met with also lying free in the ground cytoplasm or embedded in non-membrane-bound patches of a fairly opaque substance (Figure). From the perusal of the pertinent literature it became obvious to us that this striking finding had already been observed by some other authors, who had studied the ultrastructural alterations in the central or peripheral nervous system of GLD patients prior to us[5–7]. In one paper it had even been emphatically stated that the cytoplasmic inclusions seen in a nerve biopsy from a child with GLD were never membrane-bound[8].

Till now, two different enzymes have been shown in human GLD to be consistently reduced in activity. The earlier detected one is lipid sulfotransferase, a biosynthetic enzyme which catalyzes the conjugation of galactocerebrosides with sulfate groups to form sulfatides. The other and later detected one is galactocerebroside β-galactosidase, a degradative enzyme which catalyzes the splitting of galactose from galactocerebrosides to form ceramides[9]. However, sulfotransferase deficiency was found to be largely restricted to the brain[3,9], whereas reduced activity of galactocerebroside β-galactosidase could be demonstrated in the CNS as well as in a variety of non-nervous tissues[3,10]. Moreover, galactocerebroside β-galactosidase could recently be shown to be partially deficient in cells and blood serum from heterozygous carriers of GLD[10]. All this together would suggest that the defect of this specific galactosidase is more likely to be the primary, genetically determined cause of Krabbe's disease. Nevertheless, lowered lipid sulfotransferase activity in the brain must be regarded also as an integral feature of human GLD[9].

The fact that cytoplasmic galactocerebroside deposits may occur, at least in some cases and/or in certain stages of human GLD, both inside and outside the lysosome system, seems, at first glance, to be incompatible with the general concept of inborn lysosomal storage diseases. In order to explain this morphological finding without

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