The Effect of α-Tocopherol on Cerium Induced Changes in Drug and Lipid Metabolism of Rat

The light lanthanons, including cerium (Ce), are known to produce a severe liver injury in rats, similar to that caused by carbon tetrachloride (CCl_4)[1,2]. Both of these hepatotoxins cause a fatty liver accompanied by changes in the structure of the endoplasmic reticulum and impairment of the drug metabolizing capacity of the liver[1,3]. The toxicity of CCl_4 is assumed to be due to reactive metabolites which initiate the destructive lipoperoxidation in the endoplasmic reticulum[4]. This effect can be prevented by administration of various antioxidants[5,6]. These have been suggested to prevent the formation of lipoperoxides and other possible toxic agents such as aldehydes or epoxy compounds[7].

The mechanism by which lanthanons cause their effects on the liver function is not known. To find out whether the same mechanism as in the case of CCl_4 could account for the toxicity of lanthanons, we treated rats simultaneously with Ce and a known antioxidant, α-Tocopherol (TP), in order to cause a possible protection against the Ce-induced impairment of the liver function.

Materials and methods. Male Sprague-Dawley rats weighing 180–200 g were used. They were divided into 4 groups: Group I (7) controls, group II (6) received Ce i.v. 2 mg/kg as chloride in physiological saline, group III (6) was treated with α-Tocopherol acetate (Roche) suspended in physiological saline 100 mg/kg i.p. daily for 5 days and group IV (6) was treated with TP as in group III and received on the 3rd day Ce as in group II. The animals were killed 3 days after Ce injection when the liver injury, according to our earlier studies[3], is maximal. Livers were removed and homogenized in 4 volumes of 0.1 *M Tris*-HCl buffer, pH 7.4, containing 1.15% KCl. The postmitochondrial fraction ($105{,}000 \times g$) resuspended in the same buffer was used for the studies of drug metabolizing activity. These included the estimations of 3,4-benzpyrene hydroxylase (BPH)[8], glucose-6-phosphatase (G-6-P)[9], uridine diphospho glucuronyl transferase (UDPGT)[10], cytochrome P-450 (Cyt P-450)[11] and NADPH-cytochrome C reductase (NADPH-Cyt-C-red)[12] activities. The activity of the soluble enzyme glucose-6-phosphate dehydrogenase (G-6-P DHG) was measured from the $105{,}000 \times g$ supernatant[13]. To study the changes in lipid metabolism, the triglyceride (TG) contents of plasma and liver[14] and the free fatty acids (FFA) in plasma[15] were measured.

Results and discussion. The activities of BPH, G-6-P and UDPGT in different groups are shown in Table I. Ce (group II) significantly ($p < 0.001$) inhibited the activities of BPH and G-6-P. This effect was also seen in animals treated simultaneously with TP (group IV). Compared with the respective control groups I and III, the significance of inhibition was only decreased in the activity of G-6-P after TP treatment. We have earlier reported that Ce activates the UDPGT[16]. In this study we found the activation even more pronounced after the pretreatment with TP ($p < 0.01$). It is possible that the Ce activated UDPGT is sensitive to the destructive effects of lipoperoxides formed during the incubation in vitro[17]. The antioxidant may keep the UDPGT activity at its high level by preventing the formation of these destructive lipoperoxides.

Table. I. Effect of α-Tocopherol on cerium induced changes in microsomal enzyme activities of rat liver

	3,4-BPH activity relat. fluorescene units/g/min	G-6-P activity (μg P_i liberated/g/min)	UDPGT activity (μmol *p*-nitrophenyl glucuronide/g/min)
Group I Control	370 ± 82	36.4 ± 4.8	1.16 ± 0.15
Group II Cerium	97 ± 48 $p < 0.001$	22.8 ± 2.2 $p < 0.001$	1.30 ± 0.20 n.s.
Percent of control	26.2	62.6	112.1
Group III α-TP	313 ± 50	30.2 ± 3.3	0.89 ± 0.33
Group IV α-TP + Ce	107 ± 32 $p < 0.001$	24.7 ± 3.8 $p < 0.05$	1.49 ± 0.19 $p < 0.01$
Percent of group III	34.2	81.7	167.4

Table. II Effect of α-Tocopherol on cerium induced changes in the activities of cytochrome P-450, NADPH-cyt C-reductase and glucose-6-phosphate dehydrogenase of Rat Liver

	Cyt P-450 (O.D. 450–500 nmg/liver)	NADPH-Cyt-C-red (O.D. 550 nm/min/g liver)	G-6-P DHG (Bücher units/g liver)
Group I Control	0.432 ± 0.099	1.40 ± 0.27	30.0 ± 7.0
Group II Cerium	0.149 ± 0.033 $p < 0.001$	0.54 ± 0.25 $p < 0.001$	78.0 ± 9.0 $p < 0.001$
Percent of control	34.5	38.6	260.0
Group III α-TP	0.349 ± 0.091	1.45 ± 0.49	29.0 ± 3.0
Group IV α-TP+Ce	0.257 ± 0.083[a] n.s.	1.33 ± 0.64[b] n.s.	64.0 ± 12.0[c] $p < 0.001$
Percent of group III	73.6	91.7	220.7

Group IV is significantly different from group II: [a] $p < 0.01$; [b] $p < 0.05$; [c] $p < 0.05$.

[1] R.O. ECKNAGEL, Pharmac. Rev. *19*, 145 (1967).
[2] G. MAGNUSSON, Acta Pharmac. toxic. *20*, suppl. 3 (1963).
[3] P. ARVELA and N.T. KÄRKI, Experientia 27, 1189 (1971).
[4] R.O. RECKNAGEL and A.K. GHOSHAL, Lab. Invest. *15*, 132 (1966).
[5] N.R. DI LUZIO and F. COSTALES, Expl molec. Path. *4*, 141 (1965).
[6] C.H. GALLAGHER, Nature, Lond. *192*, 881 (1961).
[7] N.R. DI LUZIO, Lab. Invest. *15*, 50 (1966).
[8] R. KUNTZMAN, L.C. MARK, L. BRAND, M. JACOBSEN, M. LEVIN and A.H. CONNEY, J. Pharmac. exp. Ther. *152*, 151 (1966).
[9] A.E. HARPER, in *Methods of Enzymatic Analyses* (Ed. H.V. BERGMEYER; Academic Press, New York 1963), p. 788.
[10] P.TH. HENDERSON, Life Sci. *9*, 511 (1970).
[11] T. OMURA and R. SATO, J. biol. Chem. *239*, 2370 (1964).
[12] B.S.S. MASTERS, C.H. WILLIAMS and H. KAMIN, in *Methods in Enzymology* (Eds. R.W. ESTABROOK and M.E. PULLMAN; Academic Press, New York 1967), vol. 10, p. 565.
[13] G.W. LÖHR and H.D. WALLER, in *Methods of Enzymatic Analyses* (Ed. H.V. BERGMEYER; Academic Press, New York 1965), p. 744.
[14] M.E. ROYER and H. Ko, Analyt. Biochem. *29*, 405 (1969).
[15] D.L. TROUT, E.H. ESTES and S.J. FRIEDBERG, J. Lipid Res. *1*, *199* (1960).
[16] P. ARVELA and N.T. KÄRKI, Acta pharmac. toxic. *31*, 380 (1972).
[17] A.K. GHOSHAL and R.O. RECKNAGEL, Life Sci. *4*, 2195 (1965).

Table III. Effect of α-Tocopherol on cerium induced changes in the lipid metabolism of the rat

	Plasma TG (μg/ml)	Liver TG (mg/g)	Plasma FFA (μeq/ml)
Group I Control	63.0 ± 26.0	5.1 ± 0.5	0.571 ± 0.067
Group II Cerium	70.0 ± 48.0 n.s.	15.0 ± 6.4 $p < 0.01$	0.890 ± 0.216 $p < 0.01$
Percent of control	111.1	294.1	155.9
Group III α-TP	61.0 ± 10.0	5.7 ± 2.5	0.727 ± 0.254
Group IV α-TP + Ce	67.0 ± 40.0 n.s.	7.3 ± 3.7[a] n.s.	0.691 ± 0.168 n.s.
Percent of group III	109.8	128.1	95.1

[a] Group IV is significantly different from group II, $p < 0.05$.

The levels of Cyt P-450, NADPH-Cyt-C-red and G-6-P DHG in different groups are presented in Table II. The TP treatment abolished almost completely the impairing effect of Ce on the activities of Cyt P-450 and NADPH-Cyt-C-red. Both of these enzymes are involved in the microsomal electron transport chain which function also in the oxidation of fatty acids[18]. This function may somehow leave these enzymes more susceptible to the destructive effects of lipoperoxidation, emphasizing thus the protective activity of the antioxidant. Although G-6-P DHG activity is enhanced by Ce treatment as reported earlier[16], the TP treatment tends to decrease this effect slightly ($p < 0.05$). The activation remains still highly significant after TP treatment ($p < 0.001$). The lack of a more pronounced antioxidant effect may be due to the soluble character of this enzyme.

The changes in lipid metabolism are collected in Table III. Ce had no significant effect on plasma TG level, but in liver the TG concentration increased threefold after Ce injection. TP alone did not alter the TG level of plasma or liver but it inhibited almost totally the Ce induced accumulation of liver TG. The same normalizing effect of TP could be seen in the increase in the plasma FFA.

Administration of CCl_4 blocks the secretion of hepatic TG into the plasma accompanied by a decrease in plasma level[19]. Our study indicates that Ce may act on some different mechanism resembling that of ethanol which leaves the plasma TG at normal or elevated level[7]. The rise in the plasma FFA concentration may be related to the catecholamine depletion of the adrenal glands caused by Ce[16], but why this elevation is inhibited by TP remains unknown[20].

Zusammenfassung. Durch Vorausgabe von α-Tocopherol wird bei der Ratte die durch Cerium bewirkte Verminderung der Glucose-6-Phosphatase-Aktivität und des Gehaltes an Cytochrom P-450, NADPH-Cytochrom C-Reduktase und Triglycerid in der Leber verhindert.

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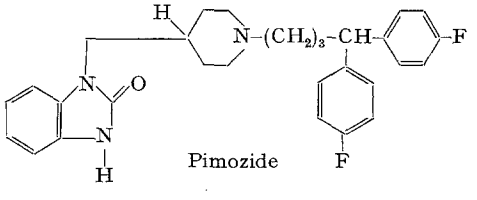
[18] S. ORRENIUS, G. DALLNER and L. ERNSTER, Biochem. biophys. Res. Commun. *14*, 329 (1964).
[19] B. LOMBARDI, Lab. Invest. *15*, 1 (1966).
[20] Acknowledgments. The work was supported by a grant from The Finnish Culture-Foundation, Finland.

Diuretic Effects of Intraventricularly Injected Noradrenaline and Dopamine in Rats

Since biogenic amines do not penetrate blood-brain barrier, we have studied behavioural effects of these amines after their application into the lateral ventricle of the rat brain[1,2]. During these studies we have incidentally observed that noradrenaline (NA) affects the diuresis in rats. The aim of this paper was to study the effects of NA and dopamine (DA) injected intraventricularly on the diuresis in rats.

Methods. Experiments were carried out on female Wistar rats weighing of 190–240 g, from Central Animal Farm of Silesian School of Medicine. Animals were divided into groups and treated as follows. I. Artificial cerebrospinal fluid (ACSF[3] injected intraventricularly (i.vt.)



Pimozide

in volume of 10 μl. II. DA, 100 μg i.vt. III. Pimozide, 5 mg/kg i.p., 2 h later 10 μl of ACSF. IV. Pimozide, 5 mg/kg i.p., 2 h later DA, 100 μg i.vt. V. NA, 100 μg i.vt. VI ACSF i.vt., 10 min later phentolamine, 100 μg i.vt. VII. Phentolamine, 100 μg i.vt., 10 min later NA, 100 μg i.vt. VIII. Normetanephrine, 100 μg i.vt.

The following substances were used: dopamine hydrochloride (Sigma); pimozide (Janssen); l-arterenol bitartrate monohydrate (NA) (Sigma); phentolamine hydrochloride (Regitine – Ciba); normetanephrine hydrochloride (Calbiochem).

All substances injected intraventricularly were dissolved in artificial cerebro-spinal fluid described by PALAIČ et al.[3], and applied in a volume of 10 μl. Injections were made into the right lateral ventricle of brain according to HERMAN[1]. Pimozide was dissolved in physiological saline solution and injected i.p. in a volume of 1 ml/kg of body weight. Immediately after i.vt. injection of drugs, rats were placed for 24 h in metabolic cages and the volume of urine was measured 2, 4 and 24 h after drug injection into the ventricle. During this time the rats had free access to water. The volume of urine was calculated per

[1] Z. S. HERMAN, Psychopharmacologia *16*, 369 (1970).
[2] Z. S. HERMAN, Intra-Sci. Chem. Rept. *8*, 85 (1974).
[3] D. PALAIČ, I. H. PAGE, P. A. KHAIRALLAH, J. Neurochem. *14*, 63 (1967).