EFFECTS OF p-TERT-BUTYLPHENYL TRANS-4-GUANIDINOMETHYL CYCLOHEXANE CARBOXYLATE HYDROCHLORIDE (NCO-650) AND ITS METABOLITE, p-TERT-BUTYLPHENOL (BP) ON DRUG-METABOLIZING ENZYMES AND FINE STRUCTURE IN RAT LIVER

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Abstracts—The effects of p-tert-butylphenyl trans-4-guanidinomethylcyclohexane carboxylate hydrochloride (NCO-650) and its metabolite, p-tert-butylphenol (BP), on the drug-metabolizing enzymes and fine structure in the rat liver were examined. Aminopyrine demethylase activity was inhibited by the administration of NCO-650 and BP at a dose of 2 and 10 mg/kg, p.o. The increases of microsomal cytochrome b and cytochrome P-450 contents were noticed at 1, 12 and 24 hr after NCO-650 and BP administration. Ascorbate-dependent lipid peroxidation of mitochondria and microsome increased by the administration of NCO-650 and BP, but NADPH-dependent lipid peroxidation decreased by these drugs. In the morphological observations of fine structure in the liver, NCO-650 and BP caused the swelling and decrease of rough endoplasmic reticulum and the increase of smooth endoplasmic reticulum. The morphological changes of liver fine structure were related to the changes of drug-metabolizing enzymes in the liver by the administration of NCO-650 and BP, which may be suggest the transitory and functional responses of these drugs in the liver. The effect of BP on the drug-metabolizing enzymes and fine structure in the liver was similar to that of NCO-650.

Key words: Hepatotoxicity, drug-metabolizing enzymes, liver fine structure, tranexamic acid derivatives.

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INTRODUCTION

p-Tert-butylphenyl trans-4-guanidinomethylcyclohexane carboxylate hydrochloride (NCO-650) of tranexamic acid derivative has been developed as a drug for antiallergics. It is known that the application of NCO-650 causes diverse membrane effects such as inhibition of chemical mediator release from the mast cells, protection of hypotonic hemolysis in the erythrocytes and increase in fluidity of dipalmitoyl-phosphatidylcholine bilayer membranes (Akagi, et al. 1983 and Arakawa, et al. 1984). We have previously reported that NCO-650 caused the induction of drug-metabolizing enzymes and the changes of fine structure in the liver (Nakayama, et al. 1985).

On the other hand, it has been considered that NCO-650 is metabolized to both metabolite, p-tert-butylphenyl(BP) and trans-4-guanidinomethylcyclohexane carboxylate hydrochloride (GMCH), by esterase in the intestine and liver. Arakawa et al. (1984) reported that the effect of BP on diverse membrane may be strong than that of GMCH. The influences of NCO-650 on the drug-metabolizing enzymes and fine structure in the liver may be due to BP which its positive metabolite. In the present study, it was designed to examination that the effect of NCO-650 on the liver may be caused by BP, and the effect of NCO-650 was compared with BP.

MATERIALS AND METHODS

Animals and drugs: Male Sprague-Dawley rats 7 weeks of age weighing approximately 200 g were used in the experiment, and they were given food and water ad libitum.

NCO-650 and BP were obtained from Nippon Chemipha Co. Ltd. (Tokyo). NCO-650 and BP were given by the gavage at a dose of 2 and 10 mg/kg.

Determination of drug-metabolizing enzyme activities: Microsomal drug-metabolizing enzyme activities and mitochondrial and microsomal lipid peroxidation in the liver examined with regard to time-course of 30 min to 72 hr after the administration of test drugs. All animals were fasted for 18 hr before sacrifice. Animals were sacrificed by dislocation of vertebrae cervicales. The abdomen was opened by a median incision, and the portal vein was cannulated with polyethylene tube. The liver were well-perfused with a ice-cold saline, and then removed. Perfused livers weighing 3 g were homogenized with 7 parts of ice-cold 1.15% KCl-0.01 M phosphate buffer (pH 7.4) in a glass-teflon homogenizer. The homogenate was centrifuged at 10,000×g for 20 min in a refrigerated centrifuge (M-160, Sakuma Co. Ltd. Tokyo). The sediment was resuspended with 6 ml of 1.15% KCl-0.01 M phosphate buffer (pH 7.4) in a homogenizer, and the supernatant of 800 ×g for 10 min recentrifuge was collected as mitochondrial fraction. A part of 10,000×g centrifuged supernatant was recentrifuged at 105,000×g for 60 min in an ultracentrifuge (Hitachi 55-2P, Hitachi Co. Ltd. Tokyo), and the pellet was collected as microsomal fraction.

Aminopyrine demethylase activity of 10,000×g centrifuged supernatant was estimated by determination of the amount of formaldehyde formed according to the methods of
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Mazel (1972) and Nash (1953). Cytochrome b5 and cytochrome P-450 contents in the microsome were determined by the method of Omura and Sato (1964). Lipid peroxidation in the mitochondria and microsome was determined by the method of Wills (1969) using ascorbic acid and NADPH as an initiator. Protein contents of mitochondrial and microsomal fractions were determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Morphological observations of fine structure in the liver: The fine structure of the liver was examined using the transmission electron microscope (TEM) according to the following procedure. The liver was removed and placed in 2% glutaraldehyde. Small sections of these samples were post-fixed in 1% osmium tetroxide and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with the TEM (Hitachi Co. Ltd. Tokyo).

RESULTS

Liver weight/body weight ratio and protein contents in the liver: Changes in the liver weight/body weight (L/B) ratio are shown in Fig. 1. The L/B ratio was elevated at 1 hr after the administration of NCO-650 and BP at a dose of 2 mg/kg. During 3 to 12 hr after NCO-650 and BP administration were lowered the L/B ratio, but recovered a control level at 72 hr after administration. The changes of L/B ratio by the administration of NCO-650 and BP were depended on the increase or decrease of liver weight. The protein contents of mitochondria and microsome in the liver were not significantly increased by the administration of NCO-650 and BP (data not shown).

Drug-metabolizing enzymes: The decreases of aminopyrine demethylase activity in the liver were noticed at 30 min to 12 hr after NCO-650 and BP administration. The activity recovered gradually, and then increased more than control level by the administration of NCO-650 and BP at a dose of 2 mg/kg (Fig. 1).

The cytochrome b5 content increased after the administration of NCO-650 and BP, and showed both peak at 1 and 12 to 24 hr after administration. The increase of cytochrome P-450 content was noticed at 12 or 24 hr after the administration of NCO-650 and BP, and both peak at 1 and 12 to 24 hr was found by those drugs at a dose of 2 mg/kg (Fig. 2).

Lipid peroxidation in the liver: Changes of lipid peroxidation in the mitochondria and microsome are shown Fig. 3 and 4. Ascorbate-dependent lipid peroxidation in the mitochondrial and microsomal fraction increased at 30 min to 6 hr after the administration of NCO-650 and BP in a dose of 2 and 10 mg/kg. In contrast, NADPH-dependent lipid peroxidation in the mitochondria and microsome decreased at 6 to 24 hr after the administration of NCO-650 and BP.

Morphological observation of fine structure in the liver: Morphological observation of fine structure in the liver using TEM are shown in Photos. 1 to 4. The swelling of rough endoplasmic reticulum (rER) and increase of smooth endoplasmic
Fig. 1. Effects of NCO-650 and BP on the liver weight/body weight ratio and liver aminopyrine demethylase activity in rats. Each point represents the mean ± S. E. of 4 rats.

*, △ and ●: Significantly different from the control (0 time).
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Fig. 2. Effects of NCO-650 and BP on the microsomal cytochrome b₅ and cytochrome P-450 contents in rat liver. Other explanations as in Fig. 1.
Fig. 3. Effects of NCO-650 and BP on the ascorbate-dependent lipid peroxidation in rat liver. Other explanations as in Fig. 1.
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**Initiator: NADPH**

Fig. 4. Effects of NCO-650 and BP on the NADPH-dependent lipid peroxidation in rat liver. Other explanations as in Fig. 1.
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Photo. 1. Electron micrograph of the rat liver at 6 hour after the administration of NCO-650 at 2 and 10 mg/kg.
A: 2 mg/kg, B: 10 mg/kg, n: nuculeus, m: mitochondria, r: rough endoplastic reticulum, s: smooth endoplastic reticulum, bc: bile canaliculi, ds: space of Disse.

Photo. 2. Electron micrograph of the rat liver at 6 hour after the administration of BP.
A: 2 mg/kg, B: 10 mg/kg.
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Photo. 3. Electron micrograph of the rat liver at 24 hour after the administration of NCO-650. A: 2 mg/kg, B: 10 mg/kg, ly: lysosome.

Photo. 4. Electron micrograph of the rat liver at 24 hour after the administration of BP. A: 2 mg/kg, B: 10 mg/kg, si: Sinusoid.
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reticulum (sER) were noticed at 6 hr after the administration of NCO-650 and BP in a dose of 2 and 10 mg/kg (Photo. 1 and 2). The slight decrease of rER and marked increase of sER were observed 24 hr (Photo. 3 and 4) and 48 hr (Photo. not shown) after the administration of NCO-650 and BP. The morphological changes of structure in the liver by NCO-650 and BP recovered almost control observations at 72 hr after those administrations.

DISCUSSION

In the previous study (Nakayama et al. 1985), we examined the changes of drug-metabolizing enzymes and fine structure in the liver by a single administration of NCO-650 at a dose of 10 to 100 mg/kg. NCO-650 caused the increase of microsomal cytochrome b\textsubscript{5} and cytochrome P-450 content and the acceleration of ascorbate-dependent lipid peroxidation. The induction of drug-metabolizing enzymes by the administration of NCO-650 was related to the morphological changes of liver fine structure such as the disarrangement of rER, detachment of ribosome from the rER and increase of sER.

In the present study, the early changes of drug-metabolizing enzymes and fine structure in liver by the administration of NCO-650 and BP were examined. NCO-650 and BP caused the decrease of aminopyrine demethylase activity and the increases of cytochrome b\textsubscript{5} and cytochrome P-450 content. The mode of induction in drug-metabolizing enzymes by NCO-650 and BP was differed from phenobarbital which shown the enzyme inhibition at 12 hr and the enzyme induction at 24 hr after administration (Nakayama, 1979).

Lipid peroxidation of mitochondria and microsome in the liver by the administration of NCO-650 and BP showed the contrary reactions by the initiator. This difference by initiator in NCO-650 and BP administered rats may be dependent on the forms of oxidation as chemically (ascorbate) (Fujita, 1973 and 1974) or enzymatically (NADPH) (Wills, 1969). The morphological changes of fine structure in the liver were observed by the administration of NCO-650 and BP, and these changes appeared later on the induction of drug-metabolizing enzymes.

The effect of BP on the drug-metabolizing enzymes and fine structure in the liver was similar to NCO-650. BP, which NCO-650 metabolite, showed the protection of hypotonic hemolysis, but GMCH, which also NCO-650 metabolite, had no effect on the erythrocytes. GMCH also had no effects on the surface tension and phase transition temperature (unpublished data). Tranexamic acid, which parent compound of NCO-650 and GMCH, had no effect on the hypotonic hemolysis in the rat erythrocytes (Arakawa et al. 1984), therefore the effect of NCO-650 on diverse membrane may be caused by BP. Furthermore, it has been known that the maximal plasma concentration of BP after the oral administration of NCO-650 in the rats was reached to 2.5-fold with that of GMCH (unpublished date). The results in the present study supported the structure-activity relationship between
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NCO-650 and BP. NCO-650 have the ability to produce cell membrane stabilization at very low concentrations. The effect of NCO-650 and BP on the drug metabolizing enzymes and fine structure in the liver may be related to the effects of those drugs on the cell membrane stabilization.

In summary, the newly developed tranexamic acid derivative, NCO-650 and its metabolite (BP), showed the induction of drug-metabolizing enzymes and morphological changes of fine structure in the liver. From results in the present study, it has been considered that the effects of NCO-650 and BP on the liver were transitory and functional responses rather than cytotoxically.

REFERENCES


