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Metabolism of Quaternary Ammonium Compounds. II.[1] Microautoradiographic Studies on the Effect of CCl_4 on the Intralobular Distribution of Tritiated BTTB in Mice Liver

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The intralobular distribution of ^{3}H-labeled *p*-biphenylmethyl-(*dl*-tropyl-α-tropinium)-bromide (^{3}H-BTTB) and the effect of CCl_4-injury on its distribution were studied in mice mainly by means of microautoradiographic technique.

It was established that ^{3}H-BTTB was mostly concentrated in the cytoplasm of hepatic parenchymal cells and that this drug distributed homogeneously over whole the intralobular area. In CCl_4-treated mice, uptake of ^{3}H-BTTB was markedly decreased in the liver and, on the other hand, was apparently increased in the kidney. In addition, the decrease in the uptake of this drug by CCl_4-treated liver occurred widely within the liver lobules and more significant decreases were found especially in the central zones of lobule. During the course of preparation of microautoradiographic tissue sections, the degree of released radioactivity in CCl_4-treated livers was inclined to increase than that of the controls.

Introduction

In the course of studies on the metabolism of quaternary ammonium compounds, previous works by means of tracer technique have revealed that ^{3}H-labeled *p*-biphenylmethyl-(*dl*-tropyl-α-tropinium) bromide (^{3}H-BTTB) was mainly concentrated in the excretory organs such as the liver and kidney[3] and that in the hepatic cells ^{3}H-BTTB was specifically bound to lysosomes.[1]

In the present work, the intralobular distribution of this drug and the effect of CCl_4-injury on its distribution were examined by means of microautoradiographic technique.

Methods

Animals and Drugs——Male mice of ddY strain weighing 20—25 g were used. Liver injury of mice were induced by intraperitoneal injection of CCl_4 at a dose of 1 ml/kg body weight 24 hr before sacrifice. Control mice were injected intraperitoneally with physiological saline. ^{3}H-BTTB (354 μCi/mg) was injected intravenously to mice via the tail vein at a dose of 1 mg/kg body weight at 5 min or 60 min before sacrifice.

Determination of Radioactivity in Tissues——At a definite time after administration of ^{3}H-BTTB, mice were sacrificed by decapitation, livers were removed and homogenized in ice-cold distilled water in a Potter-Elvehjem homogenizer. Radioactivities in homogenates were determined with a liquid scintillation counter (Aloka LSC-501) using the scintillator described in the previous report.[1] Samples (0.1—0.5 ml) were added to 10 ml of the scintillator and the radioactivity was determined.

Microautoradiography——At a definite time after the administration of ^{3}H-BTTB, livers of mice were removed, cut into slices and immersed into 10% formalin-phosphate buffer (pH 6.8). After the fixation for 3 days, dehydration procedures were carried out in 70%, 80%, 90%, 95%, 99.5%, absolute ethanol and absolute acetone, and then the resulting slices were treated with benzene 3 times in order to remove ethanol. Thereafter these were embedded in paraffin and prepared the histological sections (about 1.0—1.5 μ thickness). Media mentioned above were subjected to the measurement of the radioactivity released from the tissue

1) Part I: Y. Echigoya, Y. Matsumoto, Y. Nakagawa, T. Suga, and S. Niinobe, *Biochem. Pharmacol.*, **21**, 477 (1972).
2) Location: *Kitashinjuku 3-20-1, Shinjuku-ku, Tokyo.*
3) T. Suga, K. Nikawa, T. Hashimoto, and H. Izayama, *Chem. Pharm. Bull.* (Tokyo), **19**, 2235 (1971).

sections. The histological sections obtained were contacted to an autoradiographic emulsion (Sakura NR-M2) on the slide glasses by a dipping method. After these sections had been exposed for 3 weeks in cold room, photographic developing was carried out and then stained according to hematoxylin-eosin methods. The "post-stained" sections were subjected to microscopic studies.

Results

Biochemical Determination of the Uptake of ^{3}H-BTTB into the Liver and Kidney

After control and CCl_4-treated mice were dosed with ^{3}H-BTTB, the radioactivities in the liver and kidney were determined biochemically (Table I). In general, the radioactivities in the kidney was higher than those in the liver. This is in good accord with the finding described in the previous report.[3]

With respect to the effect of CCl_4 on uptake of ^{3}H-BTTB, radioactivities in the liver of CCl_4-treated mice were markedly decreased to 49% of the control at 5 min and to 32% at 60 min, respectively. In the kidney, in contrast to the liver, radioactivities in CCl_4-treated mice were apparently increased to 149% of the control at 5 min although no significant change was observed at 60 min.

Table I. Effect of CCl_4 on the Distribution of Radioactivity in the Liver and Kidney after *i.v.* -Injection of ^{3}H-BTTB (Biochemical data)

Tissue	Time after the injection (min)	Radioactivity (dpm/g tissue) $\times 10^{-6}$	
		Control	CCl_4-treated (%)
Liver	5	4.1±0.2	2.0±0.3 (49)
	60	1.9±0.4	0.6±0.1 (32)
Kidney	5	6.7±0.2	10.0±0.5 (149)
	60	5.1±0.2	4.9±0.3 (96)

Values are means ± standard deviations from 6 animals.

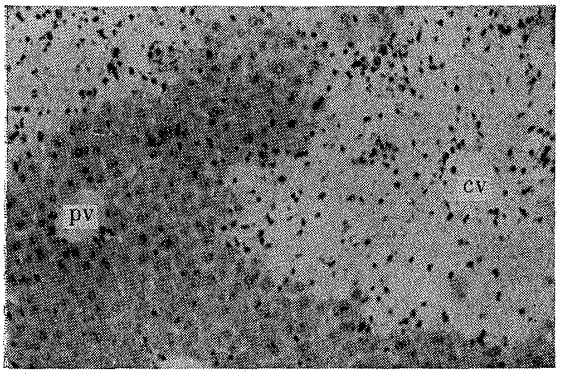

Fig. 1. Photomicrograph of Mouse Liver 24 hr after *i.p.* -Injection of CCl_4 (1 ml/kg body weight) stained with Hematoxylin-Eosin (H-E) (×280)

pv: portal vein cv: central vein

Microautoradiography of the Liver in Mice Dosed with ^{3}H-BTTB

In the liver at 24 hr after administration of CCl_4, as seen in Fig. 1, there were apparent features of toxic liver injury; there were filled with necrotic anuclear cells, disorganization of the cell cords and deposition of fat, especially in the areas of central veins. In the peripheral zones of the lobules (the areas of portal veins), on the other hand, some extent of fat deposition was observed, though neither cell necrosis nor disorganization of the cell cords was recognized.

Microscopic autoradiograms from livers of control and CCl_4-treated mice 60 min after *i.v.* injection of ^{3}H-BTTB were shown in Fig. 2. Most of silver grains were found in parenchymal cells but little was seen in Kupffer cells or sinusoidal areas. In addition, those grains were found to locate mostly in the cytoplasm of cells.

In control liver, the grains uniformly distributed in the lobules. In CCl_4-treated liver, on the other hand, the areas of central veins were found to be less in the grain number than those of portal veins.

Table II shows the distribution of radioactivities in two sides of the liver lobules of control and CCl_4-treated mice. At 5 min after administration of ^{3}H-BTTB, grain numbers in the

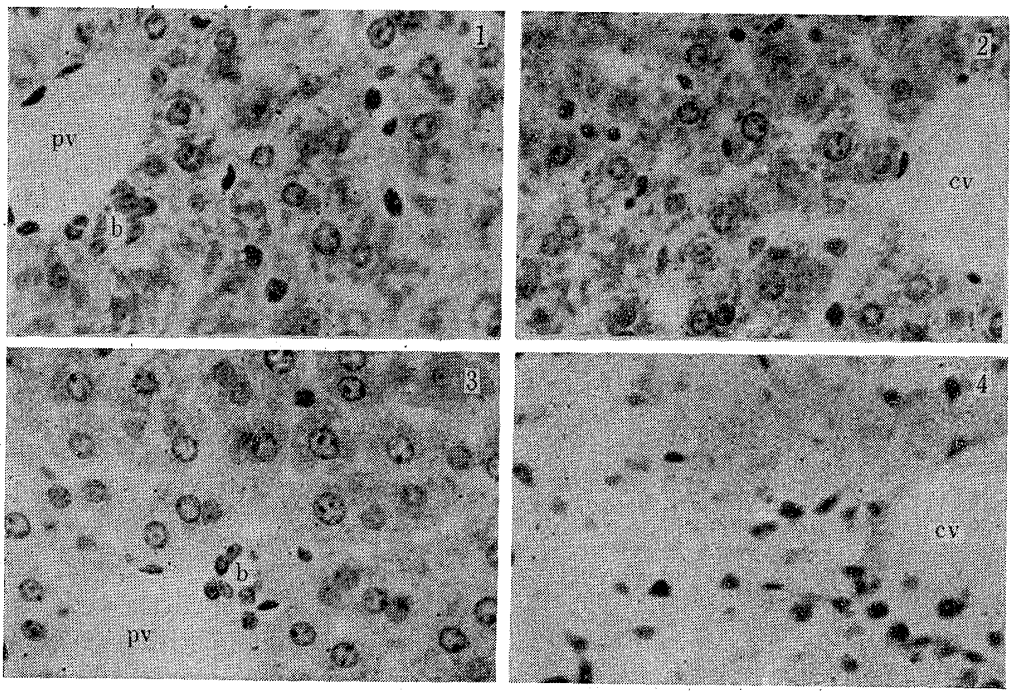

Fig. 2. Microscopic Autoradiograms of the Control and CCl_4-treated Mice Liver 60 min after *i.v.* -Injection of ^{3}H-BTTB (H-E staining, ×700—800)

1. The area around the portal vein (pv) of the control mouse liver
2. The area around the central vein (cv) of the control mouse liver
3. The area around the portal vein (pv) of CCl_4-treated mouse liver
4. The area around the central vein (cv) of CCl_4-treated mouse liver

b: bile duct

areas of central veins were apparently decreased in CCl_4-treated liver and reached 48.7% of the control, and were also decreased in the peripheral zones to reach 49.1% in spite of lower degree of necrosis in these areas. These results indicate that similar decreases in uptake of ^{3}H-BTTB occur not only in the necrotic cells but also in the relatively intact cells.

At 60 min after administration, the grain numbers in the central zones of the lobules were markedly decreased in CCl_4-treated liver and reached 20.6% of the control. On the other hand, less degree of decrease (53% of the control) was found in the peripheral zones.

Table II. Effect of CCl_4 on the Distribution of Radioactivity in the Liver Lobules after *i.v.* -Injection of ^{3}H-BTTB (Autoradiographic data)

Intralobular area	Time after the injection (min)	Radioactivity (No. of grain/cell)	
		Control	CCl_4-treated (%)
Central vein	5	2.75±0.45	1.34±0.17 (48.7)
	60	3.20±0.28	0.66±0.08 (20.6)
Portal vein	5	2.34±0.47	1.15±0.22 (49.1)
	60	2.60±0.36	1.39±0.47 (53.5)

Values are means ± standard deviations from 150 cells.

Each liver from four groups of mice was subjected to the preparation of the histological sections and radioactivities released into media during those procedures were determined. As shown in Fig. 3, considerable amounts of radioactivities were released from the sections during fixation and ethanol-treatment. Release of the radioactivity of 5 min-liver were higher than that of 60 min-liver. Especially in 5 min-liver, when treated with CCl_4, higher radioactivity (more than 85%) was liberated than the control (about 75%), although no significant change was seen between the control and CCl_4-treated in 60 min-liver.

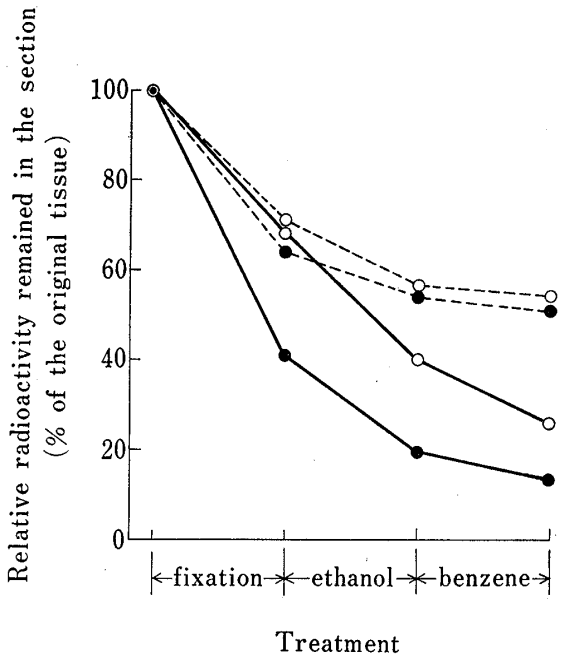

Fig. 3. Release of Radioactivity during Fixation, Ethanol- and Benzene-Treatment Procedures of Mouse Liver

Detailed procedures were seen in the "Methods.
—○—: 5 min after the injection of ^{3}H-BTTB in the control mouse
—●—: 5 min after the injection of ^{3}H-BTTB in CCl_4-treated mouse
---○---: 60 min after the injection of ^{3}H-BTTB in the control mouse
---●---: 60 min after the injection of ^{3}H-BTTB in CCl_4-treated mouse

Discussion

It was reported previously[3] that ^{3}H-BTTB injected to rats was concentrated in the liver and kidney, and that it was rapidly excreted in bile. Shindo, *et al.*[4] reported that anisotropine methbromide injected to mice was mainly concentrated in those organs. Similar results were obtained with other tropane alkaloids; methylatropine[5] and scopolamine.[6] Furthermore, it was found in the author's laboratory[1] that quaternary ammonium tropane alkaloids including BTTB specifically bound to lysosomal membrane of the liver cell.

In the present work, the intralobular distribution of this drug and the effect of CCl_4-injury on its distribution were exmined by means of microautoradiographic technique. From the results obtained by this technique, it was established that ^{3}H-BTTB was distributed homogeneously over the lobules of liver and that it located mostly in the cytoplasm of hepatic parenchymal cells.

From the results obtained by biochemical experiments, uptake of ^{3}H-BTTB to liver was markedly decreased in CCl_4-treated livers and, on the other hand, uptake of it to kidney was apparently increased at 5 min after injection of ^{3}H-BTTB. It has been known that the blood flow in the liver did not change even when animals were treated by CCl_4[7] and that no histological change was observed in the kidney. Therefore, this unexpected phenomenon is assumed to occur as results of following combined effect; blood level of ^{3}H-BTTB became higher in CCl_4-treated mice than in the control because uptake of this drug to the liver was markedly decreased in those mice and affinity of this drug for the kidney is higher than to the liver.

It has been well known that CCl_4 caused extensive centrilobular necrosis in liver.[8] With regard to intralobular distribution of ^{3}H-BTTB, the radioactivity in the area of central vein was found to show a marked decrease by CCl_4. By means of semi-quantitative analysis, an apparent decrease in radioactivity was also seen in the central zone of lobule although not so in the peripheral zones.

Autoradiographic technique has been known to possess an important defect of low quantitative accuracy because a large proportion of radioactivity was lost from tissue sections during histological procedures. The present work also can not be free from this defect and, in fact, considerable amount of radioactivity, sometimes to 85% of the original tissues, was found to be lost during the procedures. However, it is, at least, confirmable that ^{3}H-BTTB

4) H. Shindo, I. Takahashi, and E. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **19**, 513 (1971).
5) L. Albanus, A. Sundwall, and B. Vangbo, *Acta Pharmacol. Toxicol.*, **27**, 97 (1969).
6) G. Werner and H-L. Schmidt, *Hoppe-Seyler's Z. Physiol. Chem.*, **349**, 677 (1968).
7) T. Oda, *Igaku no Ayumi*, **26**, 134 (1958).
8) G.R. Cameron and W.A.E. Karumaratne, *J. Pathol. Bacteriol.*, **41**, 267 (1935).

is mainly concentrated in the cytoplasm of hepatic parenchymal cells and that, in the control liver, this drug distributes homogeneously in the intralobular area. In addition, it can be proposed by this techniques that decrease in the uptake of this drug by liver injury occurs widely within the liver lobules and that more significant decreases occur in the central zones of lobule.

During the course of preparation of microautoradiographic tissue sections, the degree of released radioactivity in CCl_4-treated liver was inclined to increase more than in the control. Although this fact may suggest that there is some difference in distribution manner between CCl_4-treated liver cells and the control ones, further investigation may be necessary for the elucidation of this problem.