Conversion of T₄ to T₃ in perfused liver of rats with carbontetrachloride-induced liver injury

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Abstract. To evaluate the effect of hepatocellular damage on thyroid hormone metabolism, the conversion of T₄ to T₃ in the perfused liver of rats with carbontetrachloride (CCl₄)-induced liver injury was investigated. The liver was perfused non-recyclicly with a synthetic medium containing 10 µg/dl T₄ for 30 min, and the T₃ production and the conversion of T₄ to T₃ were examined.

The serum T₃ level was significantly (P < 0.05) lower in liver-injury rats (72 ± 9 ng/dl) compared with controls (88 ± 10 ng/dl). The uptake of T₄ (4.15 ± 0.85 µg/g/30 min), net T₃ production (180 ± 48 ng/g/30 min), and conversion rate of T₄ to T₃ (4.4 ± 0.9%) in the perfused liver of liver-injury rats were similar to the values (3.78 ± 0.66 µg/g/30 min, 191 ± 31 ng/g/30 min, and 5.0 ± 1.0%) in control rats, whereas the release of T₃ (12.7 ± 3.2 ng/g/30 min) in the effluent in injured livers was significantly (P < 0.05) lower than that (16.9 ± 3.0 ng/g/30 min) in control livers.

We concluded that in CCl₄-induced liver-injury rats, the low serum T₃ level may partly be due to the decreased release of T₃ from liver cells after conversion of T₄ to T₃ rather than to a decreased conversion of T₄ to T₃.

The liver plays an important role in thyroid hormone metabolism being involved in their conjugation, excretion, and peripheral deiodination. Several studies have reported low serum T₃ concentrations in patients with liver disease, particularly cirrhosis of the liver, suggesting an altered T₄ metabolism in hepatic disorders (Inada & Sterting 1967; Nomura et al. 1975; Lumholtz et al. 1978; Faber et al. 1981; Borzio et al. 1983; Kabadi & Premachandra 1983). Furthermore, it has been suggested that the conversion of T₄ to T₃ is significantly reduced in patients with liver cirrhosis due to hepatocellular damage (Nomura et al. 1975; Lumholtz et al. 1978; Faber et al. 1981; Borzio et al. 1983; Kabadi & Premachandra 1983).

The present study was carried out to define the conversion of T₄ to T₃ in the perfused liver of rats with carbontetrachloride-induced liver injury.

Materials and Methods

Materials

Dextran T-70 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Bovine serum albumin (BSA, Fraction V) and L-thyroxine were purchased from Sigma Chemical Co., St. Louis, MO. T₃ (less than 0.15%) was detected by radioimmunoassay in the T₄ used in the present study (probably due to contamination or crossreaction). T₄ used in perfusions was dissolved in 0.01 N NaOH (200 µg/ml) and stored at -20°C.

Animals and induction of liver injury

Male Wistar albino rats, weighing about 100 g, were used in the present study. Liver injury was induced by the method of MacLean et al. (1969). In brief, sodium phenobarbitone was dissolved in tap or distilled water at a concentration of 0.5 g/l. This was the only drinking water available to the experimental rats. Control rats were given distilled water. CCl₄ was placed in the inhalation chamber and the rats were placed here for 10 min every Tuesday and Friday for 8 weeks. After 8 weeks, the survival rate of the rats was 40%.
Liver perfusion

A modification of the method of Hems et al. (1966) was used for isolation and perfusion of the rat liver in situ. The rats were anaesthetized with ip pentobarbital sodium (30 mg/kg) after 18-24 h fast, and the abdomen was opened through a midline incision. The intestines were then placed in the left side of the animal, and a polyethylene tube (1 French scale) was inserted into the bile duct to collect the bile.

The thin strands of connective tissue between the right lobe of the liver and the vena cava were cut, and a loose ligature was placed round the inferior vena cava above the right renal vein, the superior mesenteric and coeliac arteries, and the portal vein. Then heparin (200 IU) was injected into the inferior vena cava. The portal vein was cannulated and the perfusion pump was started. The ligatures round the portal vein and the arteries were tied. The thorax was then opened and an outflow cannula was inserted through the right atrium into the thoracic vena cava. Finally, the ligature round the abdominal inferior vena cava was tied, thus closing the circuit. The liver was perfused without recirculation with a synthetic medium that consists of a Krebs-Ringer bicarbonate buffer containing 0.5% BSA, 5.5 mM glucose, and 4.6% Dextran T-70. The flow rate was 2.0 ml/g liver weight per min in situ. The liver was pre-perfused for 15 min with the medium not containing T4, whereas it was perfused with the medium containing 10 μg/dl T4 (free T4 concentration in the perfusate 6.0 ng/dl) for the following 30 min. The venous effluent was collected every 5 min and stored at -20°C until the time of hormone assay. During perfusion, the medium and the chamber were warmed and kept at 37°C and the medium was bubbled with a mixture of 95% O2 and 5% CO2.

Preparation of homogenates

After the perfusion, samples of the liver (about 50 mg) were homogenized in 4 ml of 100% ethanol. The homogenates were then centrifuged at 1000 x g for 10 min at 4°C and the supernatant stored at -20°C for assay. With this method, the efficiency of extraction of known amount of T3 in the liver was 72 ± 9%.

T4 and T3 assay

Serum, perfusate, and liver samples were measured for T4 and T3 by radioimmunoassay. T4 was assayed by T4 radioimmunoassay kit (Nuclear-Medical Laboratories, Texas) and T3 was measured by T3 radioimmunoassay kit (Dainabot Co., Tokyo). The sensitivity of these assays was 1 ng/ml for T4 and 25 pg/ml for T3. Intra- and inter-assay coefficients of variation, respectively, were 9 and 17% for T4, and 7 and 15% for T3.

Calculations

The uptake of T4 by the liver was calculated as: (T4 infused − perfusate T4) × perfusion volume. Net T3 production was calculated as: (perfusate T3 + tissue T3) − T3 infused. The conversion rate of T4 to T3 was calculated by dividing the net T3 production by the T4 uptake in the liver. T3 content of the liver was calculated by subtracting, from the observed liver T3 concentration, the mean liver T3 concentration in a comparable group of rats following perfusion with the medium containing no T4.

Statistical analysis of data

Statistical analysis was performed using Student’s t-test. Data are presented as the mean ± SD.

Results

Serum T4, T3, GPT and GOT levels, and liver weight in liver-injury rats

As shown in Table 1, the serum T4 level (5.6 ± 1.8 μg/dl) in the liver-injury rats was similar to that (5.8 ± 1.7 μg/dl) in the controls. However, the T3 concentration (72 ± 9 ng/dl) in liver-injury rats was significantly (P < 0.05) lower than that (88 ±

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<tr>
<th>Table 1. Serum T4, T3, GPT and GOT levels, and liver weight in liver-injury rats.</th>
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<td><strong>T4</strong> (μg/dl)</td>
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<td>Control rats (n = 6)</td>
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<td>Liver-injury rats (n = 7)</td>
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The values are the mean ± sd. *P < 0.05, **P < 0.02: significantly different from control rats.
10 ng/dl) in controls. Serum GPT (48 ± 8 K-U) and GOT (242 ± 61 K-U) levels in liver-injury rats were significantly \((P < 0.02)\) higher than those (30 ± 7 K-U and 128 ± 24 K-U, respectively) in controls. Wet weight of the liver (12.7 ± 0.8 g) in liver-injury rats was similar to that (13.2 ± 0.5 g) in controls.

\(T_4\) uptake, perfusate \(T_3\), tissue \(T_3\), net \(T_3\) production, and conversion rate of \(T_4\) to \(T_3\) in perfused livers

As shown in Fig. 1, the \(T_4\) uptake (4.15 ± 0.85 \(\mu\)g/g/30 min) in the injured livers was similar to that (3.78 ± 0.66 \(\mu\)g/g/30 min) in the control livers. Tissue \(T_3\) (174 ± 50 ng/g/30 min), net \(T_3\) production (180 ± 48 ng/g/30 min), and conversion rate of \(T_4\) to \(T_3\) (4.4 ± 0.9%) in injured livers were similar to the values (184 ± 30 ng/g/30 min, 191 ± 31 ng/g/30 min, and 5.0 ± 1.0%, respectively) in controls. However, perfusate \(T_3\) (12.7 ± 3.2 ng/g/30 min) in injured livers was significantly \((P < 0.05)\) lower than that (16.9 ± 3.0 ng/g/30 min) in controls.

**Biliary excretion of \(T_4\) and \(T_3\)**

Biliary excretion of \(T_4\) and \(T_3\) was very small and negligible in both injured and control livers.

**Discussion**

Jennings et al. (1979, 1984) reported that perfused rat liver preparation was a suitable model for studying the conversion of \(T_4\) to \(T_3\). An advantage of this model is that the contributions of \(T_4\) uptake and the conversion of \(T_4\) to \(T_3\) (activity of \(T_4\)-5'-monodeiodinase) can be assessed simultaneously. In the present study, a low concentration of serum \(T_3\) was observed in rats with carbontetrachloride-induced liver injury. On the other hand, \(T_4\) uptake, net \(T_3\) production, and the conversion of \(T_4\) to \(T_3\) were not impaired in the perfused liver of \(\text{CCL}_4\)-induced liver-injury rats. Phenobarbitone is known to induce microsomal enzyme activities and may induce deiodinase activity (Rootvelt et al. 1978; Yeo et al. 1978). Carbontetrachloride leads to a loss of hepatic function and, therefore, a lowering of deiodinase activity. Since the control group did not receive phenobarbitone, the alterations in the experimental group may be the result of the combined effects of phenobarbitone and carbontetrachloride. The present in vitro results were contradictory to the results of some investigators who have shown a decreased conversion of \(T_4\) to \(T_3\) in patients with cirrhosis of the liver (Nomura

**Fig. 1.**

Uptake of \(T_4\), perfusate \(T_3\), tissue \(T_3\), net \(T_3\) production, and conversion rate of \(T_4\) to \(T_3\) in perfused livers. The bars represent SD. □ liver from control rats \((n = 6)\). ■ liver injured with \(\text{CCL}_4\) \((n = 7)\). *\(P < 0.05\): significantly different from control liver.

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et al. 1975; Lumholtz et al. 1978; Faber et al. 1981; Borzio et al. 1983; Kabadi & Premachandra 1983). This discrepancy may be due to the difference of hepatocellular damage between CCl₄-induced liver injury and cirrhosis of the human liver or differences between an in vitro and in vivo study.

The decreased release of T₃ in the effluent from the injured liver is of some interest. Although we do not know the details of the secretory mechanism of T₃ from liver cells, the present results suggest that the secretory process of T₃ from liver cells after conversion of T₄ to T₃ was impaired in the liver injured with CCl₄. In the present study, the liver was perfused with a perfusate with high concentration of T₄ (free T₄ concentration 6.0 ng/dl). Further studies are needed to clarify the mechanism of decreased T₃ release from the perfused liver of rats with CCl₄-induced liver injury.

In summary, it is concluded that in CCl₄-induced liver-injury rats the low serum T₃ level may partly be due to the decreased release of T₃ from liver cells after conversion of T₄ to T₃ rather than to a decreased conversion of T₄ to T₃.

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