EFFECTS OF 1-MORPHOLINOACETYL-2-METHYL-3-PHENYL-4-OXO-1, 2, 3, 4-TETRAHYDROQUINAZOLINE HYDROCHLORIDE (HQ-275) ON ACUTE EXPERIMENTAL HEPATIC INJURY INDUCED BY CARBON TETRACHLORIDE IN RATS

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Abstract—Pharmacological effects of HQ-275 on CCl₄-liver injury have been investigated, and the results compared with those of rats treated with CCl₄ plus HQ-275 with those of normal rats and only CCl₄-treated rats. In the excretion test of biliary flow, bilirubin, solid contents and B·S·P, rats treated with CCl₄ alone or with CCl₄ plus HQ-275 showed less excretion than those of normal rats, and the differences among these three categories were not significant. HQ-275 revealed a potent recovery process from the liver damage caused by CCl₄ in both histological and biological levels. Changes of Na⁺ and K⁺ in bile, water content of liver and body temp. were recognized among the three groups. It can be concluded from over-all results that there may be some differences between structural and functional recovery processes from CCl₄-hepatotoxicity.

In a previous report from our laboratory, 1-morpholinoacetyl-2-methyl-3-phenyl-4-oxo-1, 2, 3, 4-tetrahydroquinazoline hydrochloride (HQ-275) has been demonstrated to have a potent inhibitory effect against experimental hepatic injury in rats by carbon tetrachloride (CCl₄) with respect to histological findings (1). CCl₄ is the agent most widely used to produce experimental liver damage in laboratory animals. Due to the importance of CCl₄ hepatotoxicity as a model for liver disease and damage, it is of interest to investigate the effect of HQ-275 on this toxicity regarding hepatic transport processes that influence biliary excretion. This paper, covers a more detailed investigation of HQ-275, choleretic actions, phenoltetrabromophthaleinsulfonate (B·S·P)-excretion and some liver function using CCl₄-intoxicated rats. In addition, influence of HQ-275 on the developmental or recovery processes of hepatic injury, (administered to rats before or after CCl₄-poisoning) were also studied employing transaminase levels (s-GOT and s-GPT) and histological findings as indices to evaluate the degree of the liver lesions.

MATERIALS AND METHODS

Male rats of Wistar strain (200–250 g) were mainly used for all experiments. In the series, six animals were employed as one group. They were fed on a commercial diet
CLEA, CA-1) and water ad libitum. CCl₄, suspended as a 10% solution in olive oil, was administered to rats orally in a dose of 1 g/kg (0.64 ml/100 g in olive oil) for the purpose of creating severe hepatic injury (2). HQ-275 was dissolved in physiological saline. Six non-treated (Gr.: A), served as control, and 18 HQ-275-treated animals were challenged with CCl₄. The latter category was divided into three groups (Gr.: B HQ-275 10 mg/kg, p.o., Gr.: C 30 mg/kg, p.o. and Gr.: D 60 mg/kg, p.o., respectively). All doses of HQ-275 were administered one hr before and one hr after CCl₄ as a single oral administration for 2 days, respectively. In addition, six normal rats (Gr.: E) were prepared as control along with the other four groups. During 6–8 hr after the second CCl₄-poisoning, the animals were all sacrificed except for those utilized for investigation of the influence of HQ-275 on histological developmental and recovery processes of CCl₄ liver injury.

**Biliary excretion:** For the biliary excretion tests, the common bile ducts of the rats, anesthetized throughout with urethane (1.2 g/kg, s.c.), were cannulated centrally so that the bile could be collected via a polyethylene tube. The cystic duct was ligated and the midline abdominal incision was sutured. The animals were maintained in a relative humidity of 60% at a room temp. of 24±1°C. After the biliary outflow had reached a steady state, the drugs were administered into the femoral vein. Measurement of the biliary flow was made every 30 min after administration of the drugs (3, 4).

**Bilirubin and solid contents:** Bilirubin and solid contents which were contained in the biliary volume excreted were assayed by the modified Evelyn-Malloy's method and by the method of dry wt., respectively (5). The outflow of the bile through common bile ducts was collected in a graduated pipette. After measurements, half of the collections every 30 min were used to measure bilirubin concentration and the remaining bile was dried for 10 hr in a constant oven at 105–110°C after which each dry residue was weighed and concentration determined.

**B·S·P-excretion:** For examination of the coloring matter excretion, B·S·P was injected into the femoral vein by a single injection in a dose of 20 mg/kg over a 30-sec period and at 3, 5, 10 and 15 min after the administration of B·S·P, the concentration of B·S·P in serum was measured according to the method of Rosenthal and expressed as a percentage (6).

**Biochemical assays:** Serum samples for the estimation of transaminase levels (GOT and GPT) were obtained from blood collected from rats by excising V. Jugularis under ether anesthesia just before sacrifice. The levels of transaminases were measured using the method of Reitman and Frankel and expressed in Karmen units (7).

**Histological findings:** It is well known that there is no direct relation between two degrees of liver lesions and the serum enzyme levels, therefore histological changes in the liver were also examined with a light microscope. A portion of liver from individual animals was fixed in 10% phosphate-buffer formalin and acetone and stained with sudan III, hematoxylin and eosin for microscopical studies. The extent of the necrosis produced by CCl₄, (focal areas of centrilobular necrosis and ballooning) was measured using an Amsler type planimeter on a microscopical photograph of low magnification.
For histochemical analysis, alkaliphosphatase and acid phosphatase activity in the liver section was measured according to the method of Gomoris (8). Furthermore, appearance of glycogen in liver tissue was demonstrated by the method of PAS-reaction (9).

Relationship between administration time of HQ-275 and histological and biochemical activity: For the purpose of examining whether or not HQ-275 exerts a therapeutic action on the damaged liver and/or the preventing effect, the drug was given to rats at various time intervals before and after CCl₄ administration. In this experiment, both histological changes of the liver such as fatty and hydro tropic alterations of the cells and the changes of transaminase levels were examined. Rats were administered p.o. 30 mg/kg of HQ-275, at 1, 3 and 6 hr prior to, or 1, 3, 6 and 12 hr after an oral administration of 1 g/kg of CCl₄. Blood was collected 24 hr after CCl₄-poisoning. The serum transaminase levels were measured at various time intervals, respectively. Histological and biological assays were carried out according to the method mentioned above. It is well recognized that the effect of CCl₄ on the liver is modified by numerous factors either in the direction of aggravation or alleviation (10). The authors therefore examined the following actions among the groups of normal (Gr.: E), only CCl₄ treated (Gr.: A) and CCl₄ plus HQ-275 treated animals (Gr.: C).

Chemical analysis of Na⁺ and K⁺ in bile: Na⁺ and K⁺ in bile were determined by
means of a Hiranuma flame photometer. Estimation of the biliary Na⁺ and K⁺ concentrations was calculated from the Na⁺ and K⁺ content in bile.

Water content of liver: The water content of liver was determined by drying the tissue for 10 hr in a constant oven at 105-110°C to constant wt.

Body temp.: It has recently been shown that one alteration in biliary excretion is the fact that it is temp. dependent (11).

Statistical analysis: A statistical comparison of the data was performed employing the Student's t test. Values of P<0.05 were considered to be representative of significant differences between means.

RESULTS

Effects on biliary excretion

The results are represented in Figs. 1 and 2. It is evident that in all doses of HQ-275, biliary flow was maximum during the first 30 min. As shown in these figures, rats treated with CCl₄ alone (Gr.: A) showed a marked decrease in biliary volume, bilirubin and solid

![Graph showing biliary flow, bilirubin concentration, and solid content over time](image)

**Fig. 2.** Upper curves (solid lines) : choleretic activity as % increase of biliary flow. Middle curves (dotted lines) : bilirubin concentration. Lower curves (solid lines) : solid content concentration. [ ] : normal (Gr. : E), ○ : CCl₄ plus HQ-275 (10 mg/kg, p.o., Gr. : B), △ : CCl₄ plus HQ-275 (30 mg/kg, p.o., Gr. : C) and ■ : control (CCl₄ 1 g/kg, p.o., Gr. : A). Maximum S.E., upper curves : 14.2%, middle curves : 11.6% and lower curves : 10.8%. Each point represents the mean of six rats. Other conditions as in Fig. 1.
contents excretion when HQ-275 was administered i.v. in any dosage. As shown in Fig. 2, the rats treated with CCl, plus HQ-275 (Gr.: B) did not show an increase in biliary flow such as was seen in normal rats. When 10 mg kg of HQ-275 was injected i.v. to rats pre-treated with CCl, plus HQ-275 (Gr.: C) and to the rats treated with CCl, alone (Gr.: A) comparable effects were seen regarding excretions of bile, bilirubin and solid contents.

**B•S•P-excretion**

In the dye excretion test, concentrations of B•S•P remained in the blood after administration of B•S•P (20 mg kg, i.v.) in a single injection as seen in Fig. 3. There were significant changes in the average concentration of B•S•P in the serum at 3-15 min after administration as compared to the normal rats (Gr.: E) and only the CCl,-intoxicated rats (Gr.: A). Regarding time-courses of B•S•P-disappearances in the serum, the rate of only the CCl,-intoxicated rats was approx. 2-2.5 times later than that of normal rats. In rats treated with CCl, plus HQ-275 (Gr.: B-D, respectively) the rate was comparable to that seen in rats treated with CCl, alone (Gr.: A).

![Graph showing B•S•P-concentration in blood over time](image)

**Effects of HQ-275 against CCl,-intoxication on the liver damage**

(a) **Histological findings:** The results presented in Fig. 4 are in accord with the histological evaluation of the livers. Rats treated with CCl, plus HQ-275 (Gr.: C) did not modify to any extent the normal structural pattern of the liver (1: a-c). Rats treated with CCl, alone (Gr.: A), however, produced an intense centrilobular necrosis associated
Liver section taken from a rat treated with CCl₄ plus HQ-275 30 mg/kg, p.o. Centrilobular necrosis and ballooning degeneration is limited to small areas (I-a). Glycogen granules are seen in the central zone (I-b).

Acid phosphatase activity was observed and compared with that of control (I-c). (II-a~c) : Liver section of a rat which had been given CCl₄ alone. Note the striking centrilobular necrotic and ballooning (II-a), glycogen granule deposition (II-b) and decrease of acid phosphatase activity (II-c).

a : Necrosis  
b : Glycogen  
c : Acid-phosphatase

Fig. 4. (I-a~c) : Liver section taken from a rat treated with CCl₄ plus HQ-275 30 mg/kg, p.o. Centrilobular necrosis and ballooning degeneration is limited to small areas (I-a). Glycogen granules are seen in the central zone (I-b). Acid phosphatase activity was observed and compared with that of control (I-c). (II-a~c) : Liver section of a rat which had been given CCl₄ alone. Note the striking centrilobular necrotic and ballooning (II-a), glycogen granule deposition (II-b) and decrease of acid phosphatase activity (II-c). a : Hematoxylin and eosin, x 100. b : PAS, x 100. c : Gomori's reaction, x 100.
with hemorrhage and edema; groups of cells bordering the necrotic areas presented ballooning. The liver cells in the periportal zones were enlarged and in that area the normal structural pattern was lost (II: a). Acid or alkali phosphatase activity and the changes of glycogen granule in the liver were also examined. In rats treated with CCl₄ alone (Gr.: A), there was a slight glycogen granule deposition in the liver cells of the periportal zones (II: b), however, there was a fairly good number of glycogen granules remaining in rats treated with CCl₄ plus HQ-275 (Gr.: C) (II: b). Furthermore, acid phosphatase activity decreased in rats treated with CCl₄ alone (Gr.: C) (II: c). On the contrary, rats treated with CCl₄, plus HQ-275 (Gr.: C) showed a marked protective effect against the decrease of acid phosphatase activity in only the CCl₄-intoxicated animals (Gr.: A) (II: c). On the other hand, an effect of HQ-275 on alkali phosphatase activity was not observed.

(b) Biochemical activity: As shown in Fig. 5, administration of CCl₄ alone resulted in a marked increase in s-GOT and s-GPT levels. This increase was partially prevented by administration of HQ-275 30 mg/kg, p.o. Furthermore, rats treated with CCl₄, plus HQ-275 (Gr.: D) revealed a similar activity to that of normal rats. It is very significant for clarification of the action mechanism of HQ-275 that the biological effect of this compound was evidenced with oral administration, and that elevation in serum transaminase levels after CCl₄-poisoning was markedly prevented by an oral dosage of 30 mg/kg of HQ-275 (Gr.: C), and also partially prevented even with a small oral dosage of 10 mg/kg (Gr.: B).

![Fig. 5. Influence of HQ-275 on s-GOT, s-GPT and necrotic levels through the developmental and recovery processes of CCl₄-liver injury in rats. The necrotic areas was measured as cm² contained in a whole area (80 cm²). Each three column represents GOT-, GPT-activity and necrotic area, respectively. Each point represents the mean S.E. of six rats. *, indicates values to be significantly different from the controls (CCl₄, 1 g kg, p.o., Gr.: A) (P<0.05).](image-url)
Relationship between administration time of HQ-275 and its histological and biological activity

The results are shown in Fig. 6. Regarding the effectiveness histological degeneration (so-called necrosis and ballooning) as parallel levels to those of transaminase were seen, only the results of transaminase levels are shown in this figure. In the groups of rats administered HQ-275 during the 24 hr period prior to CCl₄-poisoning, neither the elevation of transaminase levels nor the histological alteration of hepatic cells were inhibited in contrast to the pronounced changes in the control, while in the groups of rats administered HQ-275 1-6 hr after CCl₄-poisoning, these biochemical and histological changes were inhibited markedly and, as in control, the same lesions were found unchanged.

![Fig. 6. Relationship between administration time of HQ-275 and effect on serum transaminase levels of CCl₄-intoxicated rats. CCl₄ was administered p.o. to rats in a dose of 1 g/kg. HQ-275, 30 mg/kg was also given p.o. at the time indicated. Rats were sacrificed to estimate s-transaminase levels 24 hr after CCl₄-poisoning. The left of each pair of bar graphs represents GOT-activity and the right that of GPT-activity, respectively. Each point represents the mean S.E. of six rats. Other conditions are as in Fig. 1.](image)

Water content of the liver

As shown in Table 1, there were significant differences (p < 0.05) among the normal rats, only CCl₄-treated rats and the rats treated with CCl₄, plus HQ-275 (Gr.: E, A and C, respectively). Percentage of water content of rat's liver treated with CCl₄, plus HQ-275 30 mg/kg (Gr.: C) was the lowest among these three groups.

Electrolytes in bile

Changes of Na⁺ and K⁺ in bile among these three groups are also shown in Table 1. Each ratio of the electrolyte concentration was parallel to that of water content, respectively.
As also seen in Table 1, body temp. among these three groups was significantly recognized. The mean temp. of rats treated with CCl₄, plus HQ-275 (Gr.: C) ranked in between the other two different groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Volume (mL/100 g/30 min)</th>
<th>Bilirubin (mg/dL)</th>
<th>Solid content (%)</th>
<th>Na⁺ and K⁺ (mM)</th>
<th>Water content of liver (%)</th>
<th>Body temp. (Rectum, °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCl₄ and HQ-275 (HQ-275 30 mg/kg)</td>
<td>0.24 ± 0.02</td>
<td>3.29 ± 0.20</td>
<td>2.85 ± 0.11</td>
<td>Na⁺ 105.00 ± 3.08 K⁺ 5.10 ± 0.12</td>
<td>73.8 ± 0.51</td>
<td>34.7 ± 0.43</td>
</tr>
<tr>
<td>CCl₄ alone (CCl₄ 1 g/kg, p.o.)</td>
<td>0.10 ± 0.01</td>
<td>5.51 ± 0.44</td>
<td>3.92 ± 0.33</td>
<td>Na⁺ 122.60 ± 2.00 K⁺ 5.94 ± 0.30</td>
<td>74.8 ± 0.28</td>
<td>32.4 ± 1.70</td>
</tr>
<tr>
<td>Normal</td>
<td>0.13 ± 0.005</td>
<td>8.19 ± 0.74</td>
<td>4.50 ± 0.17</td>
<td>Na⁺ 132.70 ± 2.80 K⁺ 6.46 ± 0.20</td>
<td>76.9 ± 0.25</td>
<td>37.1 ± 1.10</td>
</tr>
</tbody>
</table>

Body temp.

As also seen in Table 1, body temp. among these three groups was significantly recognized. The mean temp. of rats treated with CCl₄ plus HQ-275 (Gr.: C) ranked in between the other two different groups.

DISCUSSION

As shown in Figs. 1 and 2, there were differences observed between normal rats (Gr.: E) and those administered CCl₄, at least (Gr.: A, B and C), regarding the action of biliary excretion when an administration of HQ-275 10 mg/kg, i.v. was given. The groups of rats treated with CCl₄ alone (Gr.: A) and with CCl₄ plus HQ-275 (Gr.: B and C) did not show an increase (dose-dependently) in biliary volume as did normal rats, while the latter hastened the recovery of concentrations of bilirubin and solid contents. As this phenomenon contradicts the data shown in Fig. 2, further investigation is now underway. As control biliary volume in rats treated with CCl₄ plus HQ-275 (Gr.: B and C), one hr after the second CCl₄-poisoning, before the experiment was greater than that of rats treated with CCl₄ alone (Gr.: A) and normal rats (Gr.: E), it is presumed that the ratio of biliary excretion after HQ-275 injection did not increase to any extent. In the average volume, the constant biliary output was approx. 2.5 times as great as that of normal rats.

In the case of B·S·P-excretion test, the effects of HQ-275 in rats treated with CCl₄ plus HQ-275 (Gr.: B and C) were similar to those seen in rats administered CCl₄ alone (Gr.: A).

It is well known that there is not necessarily a relationship between the degree of protection against histological changes and the transaminase levels. Treatment of rats with CCl₄ plus HQ-275, however, markedly decreased the extent of the histological changes produced by CCl₄, which showed centrilobular necrosis surrounded by the cell ballooning, glycogen and acid phosphatase deposition. Furthermore, HQ-275 protected the liver of rats from the toxic action of CCl₄ by preventing an increase in transaminase levels.
in parallel with the case of protection against necrosis.

From the fact that a pre-administration of HQ-275 did not protect the liver from the toxicity of CCl₄ in spite of revealing a strong protective effect in cases of post-administration, it may be concluded that HQ-275 has a therapeutic effect rather than a protective one. In addition, HQ-275 was demonstrated as not acting at the early stages of the pathogenetic process of CCl₄ liver injury. Considering these results, it would appear that the action mechanism of HQ-275 on CCl₄ intoxication, would prevent degeneration of the hepatic cells which were thought to be the result of another mechanism (12) rather than the leakage of hepatocellular enzymes due to the CCl₄-induced alterations of hepatic membrane systems.

The changes of Na⁺, K⁺, bilirubin and solid contents in bile, water content of liver and body temp. between normal (Gr.: E) and CCl₄-treated animals (Gr.: A and C) were examined. Among these three groups, a relationship was markedly recognized. It is postulated that the lowest biliary content of rats treated with CCl₄ plus HQ-275 (Gr.: C) among these three groups was the result of the potent hydrocholeretic action of HQ-275 administered one hr prior to and 5 hr after CCl₄-administration.

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