EFFECTS OF 1-MORPHOLINOACETYL-2-METHYL-3-PHENYL-4-OXO-1, 2, 3, 4-TETRAHYDRO QUINAZOLINE HYDROCHLORIDE (HQ-275) ON α-NAPHTYL ISOTHIOCYANATE- AND/OR THIOACETAMIDE-INDUCED LIVER DAMAGE IN RATS

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Abstract: Pharmacological properties of 1-morpholinoacetyl-2-methyl-3-phenyl-4-oxo-1, 2, 3, 4-tetrahydro quinazoline hydrochloride (HQ-275), which has a potent choleretic activity, on ANIT- and TAA-hepatic injury have been investigated in rats for biochemical, histochemical levels and the functions in the liver. Regarding the actions of biliary and solid content excretion, HQ-275 demonstrates differences between normal rats and the groups administered ANIT and/or TAA, and also the lowered values of Al-P, transaminases and bilirubin in plasma, elevated by the ANIT-poisoning. Thus a comparable effect of HQ-275 against TAA-poisoning was not observed. TAA-induced elevation of the plasma-lipid level was significantly prevented by the administration of HQ-275. Moreover, under light microscopical examination, the decrease of 5'-N by the ANIT-poisoning was prevented even with small doses of HQ-275 3-6 mg/kg, p.o. Elevation of the cholesterol level in plasma and decrease of ATP-ase in histochemical findings by ANIT-intoxication were not in themselves recognizable. Changes of Na+ and K+ in bile and water content of liver by poisoning of ANIT were clearly prevented with HQ-275. On the other hand, HQ-275 revealed no effects on the actions produced by TAA-intoxication except for those mentioned. Such being the case it can be concluded that HQ-275 inhibits the ANIT-induced hepatic injury to a greater extent than the TAA-induced hepatic damage for biochemical and histochemical levels.

In a previous report from our laboratory, l-morpholinoacetyl-2-methyl-3-phenyl-4-oxo-1, 2, 3, 4-tetrahydroquinazoline hydrochloride (HQ-275) was demonstrated to have a potent inhibitory effect against experimental hepatic injury produced by carbon tetrachloride (CCl4) in rats with respect to both histological findings and transaminase levels (1-4). It is also well known that α-Naphtyl Isothiocyanate (ANIT) and Thioacetamide (TAA) are also agents which produce experimental liver damage in laboratory animals in addition to CCl4 and ethionine. For example, a single dose of ANIT results in elevation of transaminases (s-GOT and s-GPT), alkal phosphatase (Al-P), cholesterol and bilirubin in blood and declines of ATP-ase and 5'-nucleotidase (5'-N) activities in liver cells, respectively. Furthermore, in respect to bilirubin, rats treated with ANIT produced a hepatic bile stagnation, that is obstructive icterus, due to occlusion of the common bile duct by calculus and/or cancer of the pancreas and a mild centrilobular necrosis bordering ballooning. On the other hand, hepatic changes by TAA, which result in a similar lesion can be produced by feeding thiourea, are histologically sinusoidal congestion, edematous swelling and increased mitosis (5, 6). Moreover, decrease of hepatic protein, lipids
esterase activity, and increase in hepatic water content and Al-P activity developments are recognized histochemically (7). But, in serum, Al-P activity is increased and esterase activity is reduced (8, 9). Therefore, as indices of the drugs against TAA-intoxication, transaminases, Al-P and hepatic lipid concentrations were determined by the usual method. Due to the relative importance of ANIT- and TAA-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, the same actions of HQ-275 against ANIT- and TAA-hepatotoxocities for biochemical and histochemical levels and the functions of liver using ANIT- and/or TAA-intoxicated rats.

MATERIALS AND METHODS

Male rats of Wistar-strain weighing 250-300 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. Animals were each given a single administration of ANIT (25 mg/kg, p.o.) or TAA (100 mg/kg, i.p.), suspended as a 10% solution in olive oil, for the purpose of creating severe hepatic injury. The liver damage produced by this dose, however, is invisible on a gross examination of the organ. The drug was dissolved in physiological saline. Twelve saline-treated rats, served as control (Gr.: X and Gr.: x), 48 HQ-275-treated animals were challenged with ANIT or TAA. The latter category was divided into eight groups (Grs.: A-D, HQ-275 3, 6, 10 and 30 mg/kg, p.o. for ANIT-poisoning and Grs.: a-d, 3, 6, 10 and 30 mg/kg, p.o. of HQ-275 for TAA-poisoning, respectively). All doses of HQ-275 were administered one hr before and one hr after ANIT- or TAA-intoxication, respectively. (eq., Gr. A received HQ-275 in two repeated doses of 3 mg/kg one hr before and one hr after ANIT-poisoning.) In addition, six normal rats (Gr.: Nor.) were prepared with other groups which were intoxicated with ANIT and/or TAA. During 24-26 hr after poisoning, animals were employed for the following experiments.

**Biliary excretion:** For the biliary excretion tests, the surgical procedure consisted of making a middle abdominal incision, ligating the cystic duct to prevent excretion of pancreatic juice, cannulating centrally the common bile duct of rats, anesthetized throughout with urethane (1.2 g/kg, s.c.), with polyethylene tubing as described previously (1, 2) and then closing the incision. The animals were maintained in relative humidity of 60% at room temp. of 24±1°C and body temp. during the experiment was monitored continuously (rectal thermistor probe) and maintained at 37±1°C by a heating pad placed underneath the body. After the biliary outflow had reached a steady state, the drugs were administered into the femoral vein. Measurement of the each sample of bile collected every 30-min period was made using a graduated pipette.

**Biliary solid content:** 0.2 ml of biliary flow collected in a graduated pipette was dried for 10 hr in a constant oven at 105-110°C after which the dry residue was weighed and the concentration determined.
Biochemical assays: Serum samples for estimation of transaminases (s-GOT and s-GPT), Al-P and bilirubin were obtained from blood collected from the rats by excising V. Jugularis under the slight ether anesthesia just before sacrifice. The levels of (i) serum transaminases were measured using the method of Reitman and Frankel and expressed in Karmen units (10). (ii) Bilirubin and (iii) Al-P were assayed by the modified Evelyn-Malloy's method and by the modified Kind-King's method, respectively (11, 12). The estimation of (iv) cholesterol level and measurement of (v) total lipids in liver tissue were carried out using the method of P. Zurkowski (13, 14) and J.H. Bragdon (15), respectively. Histological changes in the liver were also examined under a light microscope.

Histochemical findings: After removing the liver, small portions of the left and central lobes of individual animals were immediately fixed in 10% cold phosphate buffer calcium formalin solution. Each section of the liver tissue was stained by the two methods of Gomori and Wachstein-Meisel for microscopical studies of 5'-N and ATP-ase, respectively (16-18).

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..

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.

Body temperature: It has recently been shown that one alteration in biliary excretions is that it is temperature dependent (19). Therefore, body temp. of each animal was measured by rectal thermistor probe just before urethane anesthesia.

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.

Fig. 1. In both Grs. of ANIT-Gr. and TAA-Gr. Upper curves (solid lines) : choleresic activity as % increase of biliary flow. Lower curves (dotted lines) : solid content concentration.  and  : normal (Gr.: Nor.),  and  : ANIT (or TAA) plus HQ-275 30 mg kg, p.o., Gr. : D (or Gr. : d) and  and  : ANIT (or TAA) plus HQ-275 10 mg kg, p.o., Gr. : C (or Gr. : c). Each vertical line indicates the S.E. of six animals. *, indicates the values to be significantly different from controls (ANIT 25 mg kg, p.o. plus saline, Gr. : X) (P<, .05).
RESULTS

Effects on biliary excretion and solid content

The results are represented in Fig. 1 in which it is demonstrated that, when 10 mg/kg. i.v. of HQ-275 was administered, rats treated with ANIT plus saline and with TAA plus saline (Gr.: X and Gr.: x) showed a marked decrease in biliary excretion as compared with normal rats, HQ-275 plus ANIT+ and HQ-275 plus TAA-treated Grs. (Gr.: C and Gr.: c), respectively. The rats treated with ANIT plus HQ-275 and/or TAA plus HQ-275, however, did not show an increase in biliary flow such as was seen in normal rats, respectively. When 10 mg/kg of HQ-275 was injected i.v. to rats pretreated with ANIT and/or TAA plus HQ-275 and to the rats treated with ANIT and/or TAA plus saline, comparable effects were seen regarding excretions of bile and solid contents. Moreover, the control volumes of bile (ml/100 g body wt./30 min) excreted just before HQ-275 injection were compared, respectively (cf. Table 1)

Biochemical assays

(a) ANIT: The changes in plasma, which were in accord with the biochemical evaluation, after administration of ANIT are presented in Fig. 2. In rats treated with ANIT plus saline, the elevation of (i) s-GOT and s-GPT values were clearly recognized and favorable effects of HQ-275 for these toxicities were observed to be dose-dependent. The levels of s-GOT and s-GPT in normal rats, however, was lower than that of the group treated with ANIT plus 30 mg/kg of HQ-275 (Gr.: D). As the result of (ii) hyperbilirubinemia,
FIG. 3. Influence of administration of HQ-275 against the high s-GOT (○) and s-GPT (●) and bilirubin (◆) levels produced by ANIT-poisoning. Each vertical line indicates the S.E. of six animals. Three dotted lines show the Grs. which were not given HQ-275 at 24 hr after ANIT administration. At the 2nd day, the values of their Grs. were significantly different from their controls which were given HQ-275, respectively (P<.05).

FIG. 4. Influence of HQ-275 on Al-P, lipid, s-GOT and s-GPT levels through the developmental and recovery processes of TAA-liver injury in rats. Each fourth column represents Al-P, lipid, s-GOT and s-GPT activity, respectively. Each point represents the mean ± S.E. of six rats. *, indicates values to be significantly different from the controls (TAA 100 mg/kg, i.p. plus saline, Gr. : x) (P<.05).
the elevation in serum bilirubin level after ANIT-poisoning was prevented entirely by an oral dosage of 30 mg/kg of HQ-275 (Gr.: D) to the level of normal rats, and with even the small oral dosage of 10 mg/kg (Gr.: C) the effect was quite obvious. Furthermore, as shown in Fig. 3, HQ-275 protected the serum of rats from the toxic alteration of ANIT by HQ-275 injected 24 hr after ANIT-poisoning. The effects on transaminase levels were parallel with the case of protection against hyperbilirubinemia. The inhibitory effect of HQ-275 on the elevated values of (iii) Al-P and against hyperbilirubinemia was not so evident. On the other hand, in rats treated with a small dose of ANIT, 25 mg/kg, p.o., elevation of the (iv) cholesterol level was not at all evident as compared with normal rats.

(b) TAA: As shown in Fig. 4, administration of TAA plus saline (control, Gr.: a) resulted in a marked increase in transaminase levels and in a slight elevation of Al-P and total lipids. This marked increase of s-transaminase levels and slight elevation of Al-P value were hardly prevented even with a large dose of HQ-275 30 mg/kg, p.o. The increase of (v) total lipids level, however, was significantly prevented and rats treated with HQ-275 30 mg/kg (Gr.: d) revealed a value similar to that of normal rats.

Histochemical findings

The results presented in Fig. 5 are in accord with the histochemical evaluation of HQ-275 against ANIT-intoxication on 5’-N damage of liver tissue. Rats treated with ANIT plus HQ-275 (Gr.: A and Gr.: B) did not modify to any great extent the normal activity of 5’-N (Gr.: Nor.), and a similar activity even with a small oral dosage of 3 mg/kg (Gr.: A) was observed. In rats treated with ANIT plus saline, however, the activity of 5’-N (Gr.: X) was markedly reduced. On the other hand, a decrease of ATP-ase in histochemical findings after ANIT-poisoning was not observed.

Fig. 5. Liver sections taken from rats treated with ANIT plus saline (a, Gr.: X), ANIT plus HQ-275 3 mg kg (c, Gr.: A), ANIT plus HQ-275 6 mg kg (d, Gr.: B) and normal rats (b, Gr.: Nor.). 5’-N activity in the central zone of the liver were compared with that of control (a). Note the striking 5’-N deposition (a), however, centrilocular decrease of 5’-N activity is limited to small areas (c). Gomori’s reaction, ×100.
Water content of liver, biliary outflow in volume and body temperature

The results of these experiments are summarized in Fig. 6.

(a) ANIT: (1) Water content of liver. There were significant differences among the normal rats, saline plus ANIT-treated rats and the rats treated with ANIT plus HQ-275 (Grs.: Nor., X, A-C, respectively). Even in a group of rats treated with 3 mg/kg, p.o. of HQ-275 plus ANIT (Gr.: A) a marked difference was observed as compared with the control group (Gr.: X). As demonstrated in the figure, the percentage in rats treated with 3, 6 and 10 mg/kg of HQ-275 (Grs.: A-C) was similar to that of normal rats, but these values were significantly different from the control (Gr.: X), respectively.

(2) Biliary outflow in volume. The changes of biliary outflow in volume (ml/100 g body wt./30 min) were markedly recognized as well as (1) water content of liver among the rats treated with ANIT plus saline (Gr.: X), ANIT plus HQ-275 treated rats (Grs.: A-C) and normal rats. The ratios of biliary volumes of these groups were in reverse order against the results of (1) water content of liver.

(3) Body temp. The differences of body temp. among five groups mentioned above (Grs.: X, A-C and Nor.) did not significantly vary. Regarding body temp., similar effects were recognized between ANIT-intoxication and TAA-poisoning in each category.

(b) TAA: (1) Water content of liver. There were significant differences between the normal rats (Gr.: Nor.) and TAA-treated rats (Gr.: x and Grs.: a-d), therefore, changes in water content among the groups (Gr.: x and Grs.: a-d) were rarely observed.

(2) Biliary outflow in volume. Volume changes of biliary outflow were observed between normal rats and TAA-intoxicated rats (Gr.: x and Grs.: a-d), in addition to the changes in water content in liver mentioned above. The ratios of biliary flow among the control group and the rats treated with TAA plus HQ-275 (Grs.: a-d) were, however, comparable in each category.

Electrolytes in bile

In the sodium concentration of ANIT-intoxicated category only was there a significant difference between ANIT plus saline-treated rats (control, Gr.: X) and the rats treated with ANIT plus HQ-275 (Gr.: C).
TABLE 1. Figures are average S.E. of six animals. *, indicates the values to be significantly different from control (Gr.: X) (P<0.05).

| Treatment            | Na⁺       | K⁺        |
|----------------------|-----------|-----------|
| ANIT plus            |           |           |
| Control, saline (Gr.: X) | 172.13 ± 7.00 | 3.25 ± 0.35 |
| HQ-275 10 mg/kg (Gr.: C) | 123.29 ± 3.60* | 2.88 ± 0.19 |
| Control, saline (Gr.: X) | 125.75 ± 0.72  | 3.25 ± 0.22 |
| TAA plus             |           |           |
| HQ-275 10 mg/kg (Gr.: C) | 122.10 ± 2.67  | 3.20 ± 0.20 |
| 30 mg/kg             |           |           |
| (Gr.: d)             | 126.24 ± 4.83  | 2.96 ± 0.18 |
| Normal rats (Gr.: Nor.) | 131.68 ± 3.75  | 3.10 ± 0.31 |

DISCUSSION

As shown in Fig. 1, there were differences observed between normal rats (Gr.: Nor.) and the rats administered ANIT and/or TAA, at least (Grs.: X, C, x and c), regarding the actions of biliary excretion and the solid concentration when an administration of HQ-275 10 mg/kg, i.v. was given. In the excretion of biliary flow and the changes of solid content concentration, the groups of rats treated with ANIT and/or TAA plus saline (Gr.: X and Gr.: x) or with ANIT and/or TAA plus HQ-275 (Gr.: C and Gr.: c) showed less excretion than those of normal rats, respectively. In the former two categories, there were significant differences and choleretic activity of each category being in the order the group of rats treated with ANIT and/or TAA plus HQ-275 (Gr.: C and Gr.: c)—control rats treated with ANIT and/or TAA plus saline (Gr.: X and Gr.: x), respectively. In the group of HQ-275-treated rats the recovery of concentrations of solid contents was faster than in the control group. Control biliary outflow in volume of rats treated with ANIT and/or TAA plus HQ-275 (Grs.: A-C and a-d) was greater than that of rats treated with ANIT and/or TAA plus saline (Grs.: X and x), but less than that of normal rats, respectively. This phenomenon is comparable to the results of choleretic activity of HQ-275 on biliary excretion in both ANIT- and TAA-intoxicated rats. Moreover, in the average volume, the constant biliary output of the rats treated with ANIT plus 10 mg/kg of HQ-275 was approx. 1.3–2.6 times as great as control rats and 1/5–2/5 times as weak as that of normal rats, respectively.

As it is well known that TAA increases the value of plasma-lipid level and in the present experiments, the elevation of lipid value by TAA was also observed. Furthermore this total lipids increase was significantly prevented by the administration of HQ-275. On the contrary, though the changes of Al-P and transaminase levels by TAA-intoxication were not at all influenced by HQ-275, the elevated values of Al-P, transaminases and bilirubin in plasma by ANIT-poisoning showed a tendency of inhibition with HQ-275 administration, respectively. In light of these facts, it is presumed that there are mechanical and/or functional differences between ANIT- and TAA-poisoning as inducers of hepatic damages.

Light microscopical studies revealed that ANIT consistently caused more extensive
decrease of 5'-N in the saline-pretreated controls than that of HQ-275-pretreated rats. This was evident, at least, in a dose of 3–6 mg/kg, p.o. of HQ-275. There was therefore no difference in the rate of protection of 5'-N between the two groups (Grs.: A and B).

Among the groups of normal rats, saline plus ANIT- and/or TAA-pretreated controls and HQ-275 plus ANIT- and/or TAA-pretreated rats, there was a marked correlation between the changes of electrolyte concentration in bile and that of water content of liver, respectively.

Changes in body temp. were not recognized in two categories of ANIT- and TAA-intoxicated rats, respectively.

In view of these results it appears that HQ-275, which has a potent choleretic activity, inhibits the ANIT-induced hepatic injury to a greater extent than the TAA-induced hepatic damage for biochemical and histochemical levels.

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