CHOLERETIC ACTIVITIES OF 1-MORPHOLINOACETYL-2-METHYL-3-PHENYL-4-OXO-1, 2, 3, 4-TETRAHYDRO QUINAZOLINE HYDROCHLORIDE (HQ-275)

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In 1959, R. Charlier reported that the most potent choleretic action was found in \( p \)-hydroxyphenyl salicylamide (Driol, PHPS) among the derivatives of benzoic acid (1). Moreover, G. Bonola et al. reported that 1-morpholinoacetyl-3-phenyl-4-oxo-1,2,3,4-tetrahydro quinazoline (Moquizone, MQZ) has the most potent choleretic activity among their derivatives (2, 3). Recently, many derivatives of 1-aminoacetyl-2-alkyl-3-phenyl-4-oxo-1,2,3,4-tetrahydro quinazoline, which include basically the main structure of PHPS and MQZ, have been synthesized by K. Okumura et al. (4, 5). From the relationship between the chemical structure and the choleretic activity, it was found that 1-morpholinoacetyl-2-methyl-3-phenyl-4-oxo-1,2,3,4-tetrahydro quinazoline (hereafter referred to as HQ-275) has the most potent choleretic activity among these derivatives. In the present experiment, qualitative evaluation of the choleretic activities of quinazoline derivatives were carried out mainly in rats. As the choledochal bile of rat devoid of gall bladder, is qualitatively and quantitatively expression of the hepatic bile, it has a constitution very similar to that of humans. In addition to the choleretic action, other pharmacological studies and toxicity of HQ-275 were also compared with those of dehydrocholic acid (DHC), PHPS, \( \alpha \)-(1-hydroxy-cyclohexyl)-butylic acid (HCHB) and \( p \)-tolylmethyl-carbinol (PTMC) in rabbits, cats and dogs.

MATERIALS AND METHODS

The chemical structure of HQ-275 is shown in Fig. 1. The drugs used were dissolved in physiological saline or suspended in 0.5% CMC solution as required.

Toxicity: Both sexes of mice ICR strain (27-30 g) and rats of Sprague-Dawley strain (180-200 g) were used. In the series more than four groups of six animals were employed. After the drugs had been injected intravenously, intraperitoneally and subcutaneously, toxic symptoms were observed. LD\(_{50}\) and the fiducial limits (p=0.05) were calculated from lethality within 7 days using the
method of C.S. Weil (6). Mongrel dogs of both sexes (5–10 kg) were also used for the observation of toxic signs.

**Biliary excretion:** Male rats of Wistar King strain (160–220 g), anesthetized with urethane (1.2 g/kg, s.c.) were mainly used. The common bile duct was cannulated centrally. After the biliary outflow had reached a steady state, the drugs were administered into the femoral vein. Measurements of the biliary flow were made every 30 min after administration of the drugs (7, 8).

**Bilirubin and solid contents:** Bilirubin and solid contents obtained were assayed by the modified Evelyn-Malloy's method after intravenous administration of the drugs (9). Outflow of the bile through common bile ducts in rats, anesthetized with urethane (1.2 g/kg, s.c.), was collected in a graduated pipette. One of the collections every 30 min was used to measure bilirubin and the remaining bile was dried for 10 hr in a constant oven at 105–110°C after which each dry residue was weighed.

**Cholesterol lowering effect:** In the male rats of Wistar King strain (160–180 g) the effects of the drugs on cholesterol level in blood were examined. Rats, which had been fed on a hypercholesterol diet containing 1.8% cholesterol, 0.45% sodium cholate and 9.1% lard for 7 days, were divided into 5 groups; each group containing of 15. The first group served as control and were given a hypercholesterol diet only. The second, third, fourth and fifth were treated with HQ-275, DHC, PTMC and PHPS in 3 doses for each group, respectively. Animals were sacrificed on the 7th day after the first oral administration. Cholesterol levels were measured by means of the method of P. Zurkowski (10, 11).

**Inhibitory action on the hepatic injury produced by carbon tetrachloride (CCL4):** Male rats of Wistar King strain (180–220 g) were used. They were fed on a commercial diet and water ad libitum. CCL4, suspended in olive oil, was administered to rats orally in a dose of 1 g/kg to cause severe hepatic injury (12). Rats were divided into 3 groups. The first group served as control, and were given only CCL4. The second and the third groups were treated with HQ-275 in 2 doses of 30, 60 mg/kg and the fourth with DHC in 60 mg/kg 1 hrs before and 6 hrs after CCL4 administration, respectively. Animals were sacrificed 48 hr after CCL4. The influence of HQ-275 on histological development and recovery processes of the fatty and hydropic changes of the parenchymal cells by CCL4 liver injury were microscopically analyzed.

**Other pharmacological actions:** Since there are many pharmacological reports about quinazoline derivatives (13–18) a general study of HQ-275 was done: i.e. effects on 1) digestive tracts, 2) respiration and blood pressure, 3) carrageenin induced edema, 4) central nervous system and 5) peripheral sites in mice, rats, guinea-pig and rabbits.

**RESULTS**

1. **Toxicity**

**Acute toxic symptoms in mice:** In mice, the toxic symptoms caused by HQ-275 were similar to those of other known choleretic agents which showed slight respiratory stimulation, salivation, lachrymation and slight diarrhea with small doses and relaxation
of the extremities with large doses. Toxic signs of HQ-275 in mice are these peripheral actions and death is attributed to the respiratory disturbance accompanied by salivation. It was found that the heart continues the beat several times after cessation of respiration.

LD$_{50}$: LD$_{50}$ with fiducial limits of HQ-275, DHC and PHPS in mice and rats are shown in Table 1, in which the toxicity of HQ-275 was similar to that of PHPS and MQZ and about 4 times more than DHC. In Table 1, the subcutaneous absorbability of HQ-275 appears

| Drugs   | Animals | Administration | LD$_{50}$, mg/kg     |
|---------|---------|----------------|----------------------|
| HQ-275  | mice    | i. v.          | 386.8 (361.1-413.4) |
|         |         | s. c.          | 409.4 (379.4-442.6) |
|         |         | p. o.          | 700.2 (594.8-824.1) |
| rats    |         | i. p.          | 534.0 (452.2-632.8) |
|         |         | s. c.          | 587.5 (              )|
|         |         | p. o.          | 1707.5 (1007.5-2890.4) |
| DHC     | mice    | i. v.          | 1492.5 (1395.1-1596.9) |
|         |         | p. o.          | 3138.7 (2852.4-3453.4) |
| PHPS    | mice    | i. v.          | 475.3 (435.8-566.2) |
|         |         | p. o.          | 1049.5 (910.4-1213.2) |
| MQZ     | mice    | i. v.          | 473.2 (374.4-598.3) |
|         |         | p. o.          | 929.6 (667.6-1293.0) |

Numbers in parentheses indicate confidence limits

Fig. 2. Upper curves (solid lines): Choleretic activity as per cent increase of the biliary flows. Lower curves (dotted lines): Bilirubin concentration. These values were in respect to the basal values (100 %). HQ-275 and MQZ 10 mg/kg, i. v., other drugs 30 mg/kg, i. v. Maximum standard errors are HQ-275: 11.8%, DHC: 14.6%, PHPS: 13.8%, HCHB: 12.8%, and MQZ: 10.6 %.

Fig. 3. Dose-response curves of HQ-275 and other typical choleretic agents. Basal biliary flow of each drug was purposed as 100 %, respectively.
very rapid in both species. In addition, salivation, slight tremor and respiratory stimulation were found in dogs after injection of HQ-275 of 10-30 mg/kg, i.v.. Generally, dogs were sedated, although there was no loss of consciousness.

2. Choleretic action

Effects of the biliary excretion: The results are represented in Figs. 2 and 3. It is evident from Fig. 2 that in all drugs, biliary flow was maximum during the first 30 min.

The foregoing results show that HQ-275 is the most active drug and can display a noticeable increase of the biliary excretion, even at very small doses. A similar effect of HQ-275 on biliary excretion was found in oral administration.

Bilirubin and solid contents: Biliary solid contents increased after drug administration. As shown in Figs. 2, 4 and 5, HQ-275 did not increase the concentration of bile. Therefore, HQ-275 may be classified among the hydrocholeretic agents. The total bilirubin excreted after administration of HQ-275 (30 mg/kg, i.v.) was as great as that of HCHB, so-called cholaneretica.

Effect on the cholesterol lowering action: As shown in Fig. 6, it is commonly observed that drugs used for the purpose of choleretic action can inhibit cholesterolemia. HQ-275 showed the most potent cholesterol lowering action. Furthermore, the potency of these drugs was almost parallel to the activities of biliary excretion.

3. Action on the liver function

Inhibitory action on the hepatic injury induced by CCl₄: It was demonstrated that HQ-275 and other choleretic drugs possess therapeutic and/or protective effects on the liver injury induced by CCl₄ at 48 hrs after CCl₄, and that HQ-275 greatly influenced the inhibition of liver damages or the recovery process. Fig. 7 shows the repaired parenchymal liver cells after formation of swollen pale cells, balloon cells and necrosis caused by CCl₄.

![Graph showing total bilirubin content excreted as per cent increase in respect to the basal values (100%).](image)

**Fig. 4.** Total bilirubin content excreted as per cent increase in respect to the basal values (100%). HQ-275 and MQZ 10 mg/kg, i.v., other drugs 30 mg/kg, i.v. Maximum standard errors are HQ-275: 12.1%, DHC: 15.9%, PHPS: 14.8%, HCHB: 13.4%, and MQZ: 12.5%.
Fig. 5 Total solid contents (dry residue) excreted as per cent increase in respect to the basal value (100%).
HQ-275 and MQZ 10 mg kg, i.v., other drugs 30 mg kg, i.v., Maximum standard errors are HQ-275: 13.4%, DHC: 14.1%, PHPS: 18.6%, HCHB: 11.9%, and MQZ: 13.7%.

Fig. 6 Cholesterol lowering effects of HQ-275 and other drugs.

Fig. 7 Influence of HQ-275 and DHC on microscopical observation level through the developmental and recovery processes of CCI, liver injury in rats. CCI, was adm orally to rats in a dose 1 g kg (a CCI, only). HQ-275 (b: 30 mg kg, c: 60 mg kg) and DHC (d: 60 mg kg) were given orally 1 hr before and 6 hr after CCI, administration, respectively.
4. Other pharmacological actions

Effects on the small intestine: HQ-275 as well as PHPS and MQZ gave the slight inhibition on the Ach-, BaCl₂, and histamine-induced contraction of the isolated rabbit.

| Drugs | Doses (mg·kg⁻¹·i.p.) | No. of exper. | Average per cent depression of traverse* |
|-------|----------------------|-------------|-----------------------------------------|
| HQ-275 | 30                   | 10          | 0                                       |
|        | 60                   | 10          | 16                                      |
|        | 100                  | 10          | 39                                      |
| DHC    | 10                   | 10          | 6                                       |
|        | 30                   | 10          | 29                                      |
|        | 60                   | 10          | 67                                      |
|        | 100                  | 10          | 94                                      |
| PHPS   | 30                   | 10          | 6                                       |
|        | 60                   | 10          | 11                                      |
|        | 100                  | 10          | 22                                      |
| MQZ    | 30                   | 10          | 3                                       |
|        | 60                   | 10          | 18                                      |
|        | 100                  | 10          | 27                                      |

*1 Average % traverse with drug × 100
Average % traverse control

**Fig. 8.** Cat, 2.2 kg, anesthetized with urethane (1.2 g·kg⁻¹, s.c.). Upper curves (A): duodenal pressure, lower curves (B): common bile duct pressure during constant rate infusion of saline 0.15 ml·min⁻¹. HQ-275 30 mg·kg⁻¹, i.v. inhibits duodenal motility and relaxes the sphincter of Oddi.
intestine. The anti-contractive dose of HQ-275 against histamine contraction by 50% was $1.5 \times 10^{-4}$ g/ml and it being higher than $2 \times 10^{-4}$ g/ml against Ach- and BaCl₂-contraction. HQ-275, DHC, PHPS and MQZ hardly affected the spontaneous movement.

**Effects on the transportation of charcoal:** The average inhibitory percent traverse of test charcoal meal in mice by HQ-275 and other known choleretic drugs are presented in Table 2, in which the average inhibitory effects of HQ-275, PHPS and MQZ were somewhat less active than that of DHC.

**Relaxation of sphincter of Oddi:** Both HQ-275 and PHPS gave slightly similar relaxant activities on the sphincter of Oddi in cats, though the depression recovered after 3–5 min (19) (Fig. 8).

**Effects on the spontaneous movement of the duodenum:** HQ-275, 10 mg/kg injected intravenously, depressed the spontaneous movements of the duodenum in cats, but did not affect the basal tone (Fig. 8). The minimum dose of HQ-275 required for complete inhibition of the movement was 6 mg/kg and at this dosage the mean duration of action was 2–5 min. The effect of HQ-275 on the duodenum was found to be nearly equal to or somewhat more potent than that of PHPS and MQZ.

**Respiration and blood pressure:** In rabbits and dogs anesthetized with urethane (1.2 g/kg, s.c.), intravenous doses of more than 6 mg/kg caused a fall in blood pressure the degree depending on dosage. With a dose of 10 mg/kg of HQ-275 the blood pressure, which had been 90–110 mmHg originally, fell transiently by 10–15 mmHg immediately after the

![Fig. 9. Effects of HQ-275 and DHC on the respiration and blood pressure in rabbits.](image-url)
injection, and then returned to the control level 3-4 min after the short-lasting elevation of reflex nature. On the other hand, an increase in both the amplitude and the frequency of respiration was observed with blood pressure fall, but it lasted only approx one min (Fig. 9).

Anti-inflammatory action: HQ-275, in a dose of 200 mg/kg oral administration, inhibited the carrageenin-induced edema of the rat paw (20). The anti-inflammatory activity of HQ-275 and MQZ was 3/4-1/2 and 1/2-1/3 times of phenylbutazone by oral administration, respectively. No such action was seen with other typical choleretic drugs.

When other pharmacological investigations were done as described in the methods, no significant effects were seen in HQ-275 and other drugs.

DISCUSSION

There are three different typical choleretic drugs such as hydrocholeretic, cholaneretic, and cholekinetic. The hydrocholeretic (DHC and PHPS) mainly increase the portion of water in biliary excretion, while the cholaneretic (HCHB and PTMC) accelerate both the concentration of bilirubin and other solid contents per biliary volume. The cholekinetics (Mg-sulfate, pilocarpine and olive oil) increase contractile activity of the gall bladder, subsequently these drugs increase biliary excretion. HQ-275 showed biliary excretion in rats devoid of gall bladder and in dogs where the gall bladder had been resected. It is therefore suggested that the biliary excretion of HQ-275 is not especially affected by cholekinetic action.

As shown in Fig. 3, the choleretic activity of HQ-275 on the biliary excretion of rats was the most potent among the other typical choleretic agents.

The portion of the bilirubin and the solid contents per biliary volume, excreted after HQ-275 administration, was not so high as HCHB, but the excreted absolute value was remarkably higher than that of other hydrocholeretic agents. Thus, the effect of HQ-275 is attributed to the action of cholaneretic.

It is well known that choleretic agents possess cholesterol lowering action. Oral administration of HQ-275 markedly lowered cholesterol levels in rat's plasma, as did other drugs. The cholesterol lowering activity was almost in proportion to biliary excretion.

Many workers with CCl4 poisoning have been under investigation for liver injury. Y. Maki, M. Takeshita, S. Miyata and S. Tanaka reported that there is no question of a decrease of some enzymes in the CCl4-affected regions of the liver (21, 22). In the present experiments, CCl4 also damaged the central zone of the lobules in the CCl4-treated rats and produced formation of the swollen pale cells, balloon cells and necrosis. On the other hand, these damages caused by CCl4 were repaired dose-dependently by the administration of HQ-275. These histological findings suggest that HQ-275 may exert hepatoprotective effects indirectly with activation of some metabolic processes as reported by previous authors.

In cats and rabbits, HQ-275 slightly inhibited the spontaneous movement of the intestine and slightly relaxed the sphincter of Oddi. Potency of these actions, however, hardly affected the transportation of intestinal contents in mice.
The effects of HQ-275 on respiration and blood pressure were qualitatively similar to those of DHC and PHPS. The depressor action was more potent than that of DHC and caused slight respiratory stimulation.

Changes of blood pressure, induced by epinephrine, acetylcholine injection and electrical stimulation on preganglionic fiber of the N. splanchnicus major, was not affected by the doses of HQ-275 10-30 mg/kg, i.v. HQ-275 did not block nictitating membrane contraction induced by stimulation of preganglionic nerve of the superior cervical ganglion. These results may suggest that HQ-275 does not affect on the autonomic nervous system.

A number of pharmacological reports about quinazoline derivatives have published various actions of HQ-275. As mentioned above, anti-inflammatory action of HQ-275 which is peculiar among choleretic agents was found to be considerable.

**SUMMARY**

Choleretic and other related pharmacological activities of HQ-275 have been investigated, and results compared with those of other typical choleretic agents.

1. The biliary excretion of HQ-275 was 4-5 times as potent as that of DHC.
2. HQ-275 was about 1/1.5 times as heavy as HCHB in the concentrative ratio of bilirubin and solid contents.
3. HQ-275 was the most effective of all choleretic agents in cholesterol lowering action being 5-6 times as potent as DHC.
4. In the protection of the liver damages by CCl4 and/or the recovery process from the damages, HQ-275 was the most potent among these agents.
5. HQ-275 slightly inhibited the transportation of digestive tract contents. The inhibitory effect was 1/4 times as potent as that of DHC.
6. HQ-275 and PHPS slightly relaxed the sphincter of Oddi.
7. HQ-275 and PHPS inhibited spontaneous movement of the duodenum, but the depression disappeared within several min.
8. HQ-275 showed no effect on the autonomic nervous system.
9. Anti-inflammatory activity of HQ-275 was 3/4 to 1/2 times as potent as phenylbutazone.
10. In intravenous administration, the toxicity of HQ-275 was 4 times as potent as DHC.

It can be concluded from over-all results that HQ-275 has more potent choleretic action, higher ratio of LD50 to the dose which increases biliary flow by 50% and milder adverse effects than PHPS, MQZ and/or DHC and that HQ-275 is hydrocholeretica as well as DHC and PHPS.

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