CURARE-LIKE ACTION OF MAGNOCURARINE, ISOLATED FROM MAGNOLIA OBOVATA*

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On the pharmacological investigation of principal constituents of magnoliaoceous plants growing in Japan, there are no scientific reports except that of Sasaki (1). He succeeded in separating some substances, in a lacquer-like state, which possess curariform activity, from the alcoholic extracts of a few plants of this family, including Magnolia obvata, Thunb.

From the bark of Magnolia obvata Thunb. (Japanese name "Hohnoki") Tomita and Inubushi (2) have recently isolated a new alkaloid, named it magnocurarine and determined its chemical structure. It was found to be N-methyl coclaurine methyl hydroxide, which resembles one half of the molecular structure of d-tubocurarine (see Fig. 1). Magnocurarine is almost colorless, microscopic, prismatic crystals, melting at 200°C with effervescence and is readily soluble in water, but insoluble in most of organic solvents.

We have decided to study in some detail the properties of magnocurarine chloride (MC) and compared the curare-like action of this substance with that of d-tubocurarine chloride (TC) and of decamethonium iodide (C₁₀).

We had planned the comparative studies of the curare-like action of MC and TC but because of the lack of TC we have been compelled to use C₁₀ instead of TC in some experiments.

METHODS

MC was dissolved in water in various concentrations. Curare-like action of MC was examined in frogs (in autumn), mice and rabbits by the usual methods. These experiments were carried out at room temperature.

In frogs, MC was injected into ventral lymph sac and three or more frogs were used in each series of experiments. The onset of ataxia, limb paralysis and the duration of these symptoms were examined. The presence or absence of blocking phenomenon at the neuromuscular junction was determined by

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muscular and sciatic nerve faradization and in some experiment one leg was ligated by Claude-Bernard’s method.

In mice, the acute toxicity of MC was determined by rapid intravenous injection and by intraperitoneal injection. The LD50 in mice was evaluated. The head drop dose as well as the lethal dose were determined by means of the prolonged slow injection assay method (MC ; 0.025 mg/15sec.).

In rabbits, the head drop assay was carried out in a manner similar to that used by Holaday.

To study the effects of MC on respiration and on the contraction of the gastrocnemius muscle of rabbits elicited by electrical stimulation of the sciatic nerve, a glass tracheal cannula connected to a tambour by means of a rubber tubing was inserted into the trachea and the gastrocnemius muscle was fastened to a heavy isometric lever, the electrical impulse to be applied through the isolated end of the sciatic nerve at the rate of 18 impulses per minute, each impulse being a single shock. The contraction of the gastrocnemius muscle and respiratory movements were simultaneously recorded on a sooted kymograph paper.

RESULTS

(I) Effect on righting reaction of the frog

Apart from differences in potency, the mode of the paralysis of a frog by MC and by TC was much alike.

With small doses (0.25 mg MC or 0.02 mg TC per 10g body weight) the frog simply weakened and the righting reaction was slowed but not abolished. Mo-
Moderate doses (0.5 mg MC or 0.05 mg TC) caused abolition of this reaction but the frog was still capable of weak reflex contraction to mechanical stimuli on the skin and the respiratory movements were still visible. In larger doses (0.75 mg MC or 0.1 mg TC) all movements ceased, respiratory or otherwise, although the heart could still be seen beating through the thoracal wall. But there was a slight difference in the duration of paralysis: the paralysis by TC was somewhat rapid in onset and passed off more quickly than that due to MC. The paralysis by MC in dose of 0.75 mg and of 1.0 mg persisted for 165 and 330 minutes respectively.

(2) Neuromuscular blocking action of MC

In order to ascertain whether the muscular paralysis produced by MC was due to neuromuscular blocking or not, it is necessary to show that MC affected neither muscle nor nerve directly. We found that it has no effect on muscle, because the frog gastrocnemius muscle, fully paralysed to stimulation through its nerve, was completely responsive to direct stimulation. After ligating one leg except the sciatic nerve by the Claude-Bernard's method, 0.75 mg of MC was injected into lymph sac. In these frogs, the contraction of magnocurarinized gastrocnemius muscle caused by electric shock of the nerve was gradually depressed and 25 minutes after administration of the MC the contractile response to ordinary or to strong nerve shock disappeared completely. But no depression of the response of the drug-free gastrocnemius muscle to shock occurred even after 35 minutes following administration of the MC (see Fig. 2).

![FIG. 2. Ability of magnocurarine chloride to block the response of frog gastrocnemius to electrical stimulation of the sciatic nerve with a single shock from a Du Bois-Reymond inductorium.](image)

Cl. Bernard-Leg: the ligated leg by the Cl. Bernard's method

↓: 0.75mg/10g was injected into lymph sac

CD: Coil distance (cm)
Effect on the spinal multineuron reflex of the frog

In this experiment, one leg of the frog was ligated according to Claude-Bernard's method. After premedication of strychnine nitrate in the dose of 0.01 mg, 1 mg of MC was injected into lymph sac to one group of frogs and to another group, 3 mg of myanesin was administered instead of MC. When complete paralysis of limbs of the frogs was produced by these drugs, the sciatic nerve of the unligated leg was stimulated by a single shock. In the magnocurarinized frog, the contractile response of the gastrocnemius muscle free from the effect of the drug was observed, but in the myanesinized one, contractile response of undrugged muscle had not been observed.

Acute toxicity in mice

Solutions containing 1 mg of MC per 1 cc were injected intraperitoneally. In 2 to 5 minutes after administering lethal doses to mice, the animals became limp and unable to walk, the head fell forward, respiration stopped in additional in 3 to 5 minutes and finally cardiac activity ceased. No attempt was made to administer artificial respiration and the animals died after mild convulsion. The LD50 per 10 g body weight of mouse was 0.455 mg. Sublethal dose of MC was able to cause paralysis of limbs but its intensity varied considerably — in some animals only ataxia developed, in others abdominal positions due to paralysis of limbs resulted. This curarizing effects appeared within several minutes after injection and persisted for 40 to 50 minutes.

Head drop dose and margin of safety

Two milligrams of MC were injected in fifteen seconds into the marginal ear vein of rabbits (2.0—2.5 kg) restrained in an enclosed box, until head drop was produced. In mice 0.025 mg of MC was injected every fifteen seconds into the tail vein till head drop occurred. The head drop dose (rabbit and mouse) were determined. C10 was assayed in the same manner and its potency was

![Fig. 3. Dose-action curve of magnocurarine chloride as well as C10 by a single fast injection in mouse.](image-url)
compared with the potency of MC.

The results are summarized in the following table.

<table>
<thead>
<tr>
<th></th>
<th>Magnocurarine chloride</th>
<th>C₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i.v.)</td>
<td>Head drop dose</td>
<td>15.63</td>
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<tr>
<td></td>
<td>mg/kg</td>
<td>(Error limit 91-109)</td>
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<td></td>
<td></td>
<td>(2 mg/15 sec.)</td>
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<tr>
<td>Mouse</td>
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<td>0.22</td>
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<tr>
<td>(i.v.)</td>
<td>Head drop dose</td>
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<td></td>
<td>mg/10 g</td>
<td>(89-111)</td>
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<tr>
<td></td>
<td>(Error limit 87-113)</td>
<td>(0.025 mg/15 sec.)</td>
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<tr>
<td>Mouse</td>
<td>Lethal dose</td>
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<tr>
<td>(i.v.)</td>
<td>mg/10 g</td>
<td>(77-123)</td>
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<tr>
<td></td>
<td>(Error limit 73-127)</td>
<td>(0.005 mg/15 sec.)</td>
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<td></td>
<td>LD₅₀</td>
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<tr>
<td></td>
<td>mg/10 g /5sec.</td>
<td>0.0148</td>
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</tbody>
</table>

In the mouse experiment in which a single fast injection assay method was used, the total dose was administered in five seconds. Sublethal doses of MC and of C₁₀ were able to produce the head drop lasting for a few minutes and the minimal head drop doses of these drugs were examined.

The dose-action curves are shown in Fig. 3. Judging from these curves, duration of curarizing effect of moderate dose of C₁₀ is slightly longer than that of MC. When margin of safety for the mouse was calculated by taking the ratio of lethal dose over head drop dose in the table, it was found to be ca. 2.2 for MC and 2.6 for C₁₀. Therefore margin of safety of MC is somewhat narrow compared with that of C₁₀. Evaluating from the dose determined by a single fast injection method, margin was 2.1 for MC and 1.85 for C₁₀.

(6) Effect on the contraction of gastrocnemius muscle elicited by nerve stimuli and on the respiration of rabbit

Rabbits were anesthetized with urethane 1 g per kg of body weight, and MC was injected into marginal ear vein in five seconds. When 5 mg per kg of MC was administered into the vein, gastrocnemius muscle reaction to successive stimuli, decreased in intensity for 5 to 8 minutes. In this dose, early respiratory depression resulted, followed by stimulation of respiration persisting for 8 to 10 minutes (see Fig. 4). Regarding the early respiratory depression Li, Jacobs, Aviado and Schmidt (3) reported that in mammals with chloralose anesthesia early thoracic respiratory depression can occur following a comparatively small dose of curare which is too weak to diminish the single twitch tension of non-respiratory skeletal muscle. In the case of administration of 10 mg per kg the inhibition of the gastrocnemius twitch and the respiratory effects were more marked and the duration of these effects was longer than that caused by 5 mg dose.
FIG. 4. Ability of magnocurarine chloride to depress the response of the rabbit gastrocnemius to electrical stimulation of the sciatic nerve with a single shock from a Du Bois-Reymond inductorium.

The upper tracing shows effect on respiration. Time: in minutes

By 15 mg per kg contractile response of gastrocnemius muscle was completely abolished within 1 minute after administration and respiratory movements ceased. During the respiratory arrest, the artificial respiration was administered. In 7 to 8 minutes after the administration, the muscular response began to appear again and simultaneously respiratory movements developed spontaneously. Recovery from these curarizing effects occurred within 25 to 30 minutes and the amplitude of contraction of gastrocnemius muscle returned to normal (see Fig. 5).

By the same method, 0.1 mg of C 10 was injected. With the dose of C 10, the muscular response was completely abolished for 7 minutes, and relatively marked sparing of respiration from the effect of the drug was observed as was noted by Castillo, Phillips and Beer (4) (see Fig. 6).
CURARE-LIKE ACTION OF MAGNOCURARINE

FIG. 5. Ability of magnocurarine chloride to block the response of the rabbit gastrocnemius to electrical stimulation of the sciatic nerve with a single shock from a Du Bois-Reymond inductorium. The upper tracing shows effect on respiration. Time: in minutes

FIG. 6. Ability of C10 to block the response of the rabbit gastrocnemius to electrical stimulation of the sciatic nerve with a single shock from a Du Bois-Reymond inductorium. The upper tracing shows effect on respiration. Time: in minutes
SUMMARY

As one part of systematic studies on the coclaurine or biscoclaurine alkaloids which are found in some plants growing in Japan, we investigated the curare-like action of magnocurarine chloride, a new alkaloid isolated from Magnolia obovata by Tomita and Inubushi. The curare-like action of this substance was examined in frogs and its potency was determined in frogs, mice and rabbits by standard methods and was compared with that of d-tubocurarine chloride and C₁₀.

1. In frogs, the mode of the paralysis by magnocurarine chloride and by d-tubocurarine chloride was much alike, but the duration of paralysis by the former was somewhat longer than that by the latter. Judged by the effective dose in the righting reaction, d-tubocurarine chloride was ten times as potent as magnocurarine chloride.

2. Neuromuscular blocking action of magnocurarine chloride was ascertained in frogs by the administration of 0.75 mg per 10 g body weight in the lymph sac.

3. Magnocurarine chloride showed no depressive effect on the spinal multineuron reflex in frogs.

4. In mice, magnocurarine chloride produced paralysis of limbs, head drop, and respiratory inhibition by intraperitoneal or intravenous injection and in larger doses it caused respiratory arrest and finally cessation of cardiac activity. Head drop doses in mice and in rabbits were shown in a table above. As to margin of safety there was no marked difference between magnocurarine chloride and C₁₀, but in the duration of curarizing effect C₁₀ surpassed magnocurarine chloride.

5. In rabbits, effects of magnocurarine chloride and of C₁₀ on the contraction of gastrocnemius muscle elicited by nerve stimuli and on the respiration were examined. Five milligrams per kg of magnocurarine chloride administered into vein produced a decrease in muscle tension and early respiratory depression, followed by stimulation. With 15 mg per kg of magnocurarine chloride, muscle response was abolished completely as with 0.1 mg per kg of C₁₀, but the simultaneous respiratory arrest, which was seen with magnocurarine chloride, was not observed with 0.1 mg of C₁₀.

REFERENCES

1) SASAKI: Fukuoka Med. Journ. 14, 381 (1921)
4) CASTILLO, PHILLIPS AND BEER: Ibid. 97, 150 (1949)