Anesthesia and Recovery with Tricaine Methanesulfonate, Eugenol and Thiopental Sodium in the Carp, *Cyprinus carpio*

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**ABSTRACT.** The anesthetic effects of tricaine methanesulfonate (MS-222), eugenol (FA-100) and thiopental sodium (RABONAL) on adult carp (*Cyprinus carpio*) were examined with the recovery process. For this purpose, behavioral response and changes in respiratory rates during anesthesia and recovery were determined. All anesthetic agents were applied to carp by immersing it in the solution of the agent. Effects of water temperature (10 and 20°C) on anesthetic and recovery process were also tested. FA-100 (25 to 100 ppm) and RABONAL (200 to 300 ppm) shortened the time required to induce each anesthetic stage and delayed recovery with dose depended manner. Higher dosage of MS-222 (50 to 200 ppm) also hastened induction of anesthesia. There was no difference in recovery times between the used concentrations of MS-222, and recovery was quicker than those observed in the cases with other agents. MS-222 caused the increase in respiratory rates. On the contrary, FA-100 and RABONAL decreased respiratory rates. The high water temperature (20°C) augmented the anesthetic effects of agents and facilitated the recovery. These results reveal the quick recovery of carp when it is anesthetized with MS-222 and indicate that the criterion of anesthesia and recovery determined in this study is useful for analysing the effect of anesthetic in adult carp.—**KEY WORDS:** anesthesia, carp, eugenol, thiopental sodium, tricaine methanesulfonate.


Anesthesia by immersing the fish in an anesthetic solution is favorably used on practical advantages. Recently, with the popularity of aquarium fishes as pets, the clinical requirement for anesthesia of them is in veterinary medicine [17]. Tricaine methanesulfonate (MS-222) has received wide acceptance as an effective anesthetic for fish [14, 18, 19]. Eugenol (FA-100) was an effective anesthetic for fresh water fish [2]. MS-222 and FA-100 are commercialized and commonly used as fish anesthetics in Japan. Thiopental sodium (RABONAL) was an ultra-short acting barbiturate agent used intravenously for anesthetic induction in human [6] and mammals [12]. Because RABONAL is highly soluble in water, it is thought to be appropriate for fish anesthesia. However, there are few reports that deal with application of RABONAL to fish anesthesia.

In surgical operations of fish, it is required to recognize sure stages of anesthesia. McFarland [13] descriptively classified the behavioral changes that occured in fish during anesthesia. Furthermore, Kikuchi et al. [11] reported the diagrammatical display of behavioral change in carp, trout and yellow tail during anesthesia and recovery with 2-amino-4-phenylthiazole. The fishes used in these studies were very small in size. The rate of anesthetic induction and the depth of anesthesia were affected by factors such as temperature, pH, ionic constitution and dissolved oxygen of environmental water [13, 21, 22, 24], and size, maturity and species of fish [1, 9].

The purpose of the present study was to examine the anesthetic effects of MS-222, FA-100 and RABONAL on adult carp with the recovery process. For this purpose, be-
Table 1. Stages of anesthesia with MS-222, FA-100 and RABONAL in the carp

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal.</td>
</tr>
<tr>
<td>1</td>
<td>Sedation; Partial or total loss of reaction in response to external stimuli. Equilibrium is normal.</td>
</tr>
<tr>
<td>2</td>
<td>Partial loss of equilibrium. Erratic swimming.</td>
</tr>
<tr>
<td>3</td>
<td>Total loss of equilibrium.</td>
</tr>
<tr>
<td>4</td>
<td>Anesthesia; Loss of reflex activity.</td>
</tr>
<tr>
<td>5</td>
<td>Medullary collapse; Respiratory movements cease. Fish death.</td>
</tr>
</tbody>
</table>

Table 2. Stages of recovery from anesthesia

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Reappearance of opercular movement.</td>
</tr>
<tr>
<td>II</td>
<td>Partial recovery of equilibrium with partial recovery of swimming motion.</td>
</tr>
<tr>
<td>III</td>
<td>Total recovery of equilibrium.</td>
</tr>
<tr>
<td>IV</td>
<td>Reappearance of avoidance swimming motion and reaction in response to external stimuli, but still behavioral response is stolid.</td>
</tr>
<tr>
<td>V</td>
<td>Total behavioral recovery. Normal swimming.</td>
</tr>
</tbody>
</table>

Behavioral response and changes in respiratory rates during anesthesia and recovery were determined. Effects of water temperature (10 and 20°C) on anesthetic and recovery process were also tested.

MATERIALS AND METHODS

Fish: Three-year-old carp (Cyprinus carpio) of both sexes, weighing 700 to 750 g were purchased from a local fish farm and acclimated for 2 to 3 days in a 300 l tank with a water content of 200 l. The water was circulated at the flow rate of approximately 13 l/min. Fresh water was supplied to the tank at the flow rate of approximately 0.5 l/min. The tanks were housed in a room where natural daylight prevailed during the day. The water temperature was kept constant at 10 ± 1°C or 20 ± 1°C. The dissolved oxygen in the acclimation water were mean 7.6 (5.8–8.4) ppm at 10 ± 1°C and 7.8 (7.1–8.3) ppm at 20 ± 1°C. The water had a pH of 6.8 to 7.5.

Fish were not fed during the acclimation and experiment.

Anesthetic: Tricaine methanesulfonate (meta aminobenzoic acid ethylester methane sulfonate, MS-222®, Sankyo), eugenol (4-allyl-2-methoxyphenol, FA-100®, Tanabe) and thiopental sodium (sodium 5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate, Rabonal®, Tanabe) were used in this experiment. In each case of anesthetization, the anesthetic was dissolved in acclimation water.

The stages of anesthesia and recovery with MS-222, FA-100 and RABONAL were summarized in Table 1 and 2, respectively.

Experimental groups: A total of 90 fish were divided into 18 groups of 5 carp each. Eight groups of fish were anesthetized with either 50, 100, 150 or 200 ppm of MS-222 at 10 ± 1°C, and at 20 ± 1°C. Six groups of fish were anesthetized with either 25, 50 or 100 ppm of FA-100 at 10 ± 1°C, and at 20 ± 1°C. Other 4 groups of fish were anesthetized with either 200 or 300 ppm of RA-
**Table 3.** Effects of anesthetic concentration and water temperature on times required to induce each anesthetic stage and for each recovery stage from anesthesia with MS-222 in the carp.

<table>
<thead>
<tr>
<th>Concentration of MS-222 (ppm)</th>
<th>Stage 1 (sec)</th>
<th>Stage 2 (min)</th>
<th>Stage 3 (min)</th>
<th>Stage 4 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>19.6±1.3 (2)</td>
<td>6.2±0.6 (2)</td>
<td>6.2±0.5 (3)</td>
<td>28.4±2.5 (3)</td>
</tr>
<tr>
<td>20</td>
<td>28.4±2.5 (5)</td>
<td>1.2±0.2 (3)</td>
<td>1.2±0.2 (3)</td>
<td>1.2±0.2 (3)</td>
</tr>
</tbody>
</table>

*Mean±S.E. with number of fish in parenthesis.

**BONAL at 10±1°C, and at 20±1°C.**

**Anesthesia and recovery:** Anesthetization was carried out in a polyvinyl vessel with 25 l of an anesthetic solution. Aeration was supplied by compressed air through an air stone in a vessel. A fish of each group was taken out from the acclimatization tank by netting and immersed in an anesthetic solution. Times required to induce each anesthetic stage and opercular rates were continuously recorded.

When the opercular rates fell to less than 5 breaths per min or when exposure time to the anesthetic solution was over than 45 min observation period, the fish was gently transferred into an acclimatization tank for recovery by netting. Times required for each recovery stage and opercular rates were recorded.

**Statistical analysis:** Data on times required to induce each anesthetic stage and for each recovery stage, and on opercular rates, were subjected to analysis of variance.

**RESULTS**

**Anesthesia and recovery with MS-222:** Table 3 shows the effects of MS-222 concentration and water temperature on times required to induce each anesthetic stage and for each recovery stage. Increasing the dosage of MS-222 (50 to 200 ppm) decreased the time required to induce each anesthetic stage in both 10 and 20°C. Three fish exposed to 50 ppm of MS-222 at 10°C and 4 fish exposed to 50 ppm of MS-222 at 20°C did not reach to Stage 3 anesthesia within 45 min. All fish exposed to 50 ppm of MS-222 did not reach to Stage 4 anesthesia within 45 min. One fish exposed to 150 ppm of MS-222 at 20°C did not reach to Stage 4 anesthesia within 45 min. Mean times required to induce total anesthesia (Stage 4) were 22.8 min at 100 ppm, 10.4 min at 150 ppm and 6.6 min at 200 ppm in 10°C of water temperature, and 33.4 min at 100 ppm, 5.9 min at 150 ppm and 4.3 min at 200 ppm in 20°C. In recovery process, mean times required for total recov-
Fig. 1. Changes in opercular rates of carp during anesthesia and recovery with MS-222 at 10°C. (I) Reappearance of opercular movement; (II) Partial recovery of equilibrium with recovery of swimming motion; (III) Total recovery of equilibrium; (IV) Reappearance of avoidance swimming motion; (V) Total behavioral recovery. Each value represents the mean ± standard error of 3 to 5 fish.

Fig. 2. Changes in opercular rates of carp during anesthesia and recovery with MS-222 at 20°C. (I) Reappearance of opercular movement; (II) Partial recovery of equilibrium with recovery of swimming motion; (III) Total recovery of equilibrium; (IV) Reappearance of avoidance swimming motion; (V) Total behavioral recovery. Each value represents the mean ± standard error of 3 to 5 fish.

ey (Stage V) were not significantly different between the concentrations of MS-222 in both 10 and 20°C (P > 0.05). Three fish exposed to 50 ppm of MS-222 at 10°C recovered to Stage III immediately after transfer to fresh water. Four fish exposed to 50 ppm of MS-222 at 20°C recovered to Stage II immediately after transfer to fresh water. One fish exposed to 150 ppm of MS-222 at 20°C recovered to Stage II immediately after transfer.
to fresh water.

In fish exposed to 50 and 100 ppm of MS-222, the differences in mean times required to induce each anesthetic stage between 10 and 20°C were not significant (P>0.05). Mean times required to induce each anesthetic stage in fish exposed to 150 and 200 ppm of MS-222 were significantly (P<0.05–0.001) less in 20°C than in 10°C. Mean times required for each recovery stage in fish exposed to 150 and 200 ppm of MS-222 were also less in 20°C than in 10°C.

Fig. 1 and 2 show the changes in opercular rates of fish during anesthesia and recovery with MS-222 at 10 and 20°C, respectively. In fish exposed to high concentrations (100, 150 and 200 ppm at 10°C; 200 ppm at 20°C) of MS-222, the opercular rates increased during induction of Stage 4 anesthesia. When reached to Stage 4, the breathing became irregular and ultimately stopped. During recovery process, the opercular rates decreased and returned to normal values. In fish exposed to low concentration (50 ppm at 10 and 20°C) of MS-222, no marked changes in opercular rates were observed during anesthesia and recovery. The opercular rates of fish acclimated at 20°C were greater than those at 10°C.

**Anesthesia and recovery with FA-100:**

Table 4 shows the effects of FA-100 concentration and water temperature on times required to induce each anesthetic stage for each recovery stage. Increasing the dosage of FA-100 (25 to 100 ppm) decreased the time required to induce each anesthetic stage in both 10 and 20°C. Two fish exposed to 25 ppm of FA-100 at 10°C did not reach to Stage 4 anesthesia within 45 min. Three fish exposed to 25 ppm of FA-100 at 20°C did not reach to Stage 4 anesthesia. All fish exposed to 50 and 100 ppm of FA-100 reached to total anesthesia (Stage 4) within 45 min. Mean times required to induce Stage 4 anesthesia were 12.6 min at 50 ppm and 10 min at 100 ppm in 10°C, and 21.0 min at 50 ppm and

### Table 4

<table>
<thead>
<tr>
<th>Concentration of FA-100 (ppm)</th>
<th>Water temperature (°C)</th>
<th>Stage 1 (min)</th>
<th>Stage 2 (min)</th>
<th>Stage 3 (min)</th>
<th>Stage 4 (min)</th>
<th>Stage V (min)</th>
<th>Stage IV (min)</th>
<th>Stage III (min)</th>
<th>Stage II (min)</th>
<th>Stage I (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>2.8 ± 0.25</td>
<td>2.0 ± 0.30</td>
<td>1.3 ± 0.30</td>
<td>1.1 ± 0.30</td>
<td>1.1 ± 0.30</td>
<td>1.1 ± 0.30</td>
<td>1.1 ± 0.30</td>
<td>1.1 ± 0.30</td>
<td>1.1 ± 0.30</td>
</tr>
<tr>
<td>50</td>
<td>20</td>
<td>3.2 ± 0.25</td>
<td>2.0 ± 0.30</td>
<td>1.8 ± 0.25</td>
<td>1.2 ± 0.30</td>
<td>1.2 ± 0.30</td>
<td>1.2 ± 0.30</td>
<td>1.2 ± 0.30</td>
<td>1.2 ± 0.30</td>
<td>1.2 ± 0.30</td>
</tr>
<tr>
<td>100</td>
<td>20</td>
<td>4.0 ± 0.25</td>
<td>2.8 ± 0.40</td>
<td>2.8 ± 0.40</td>
<td>1.8 ± 0.25</td>
<td>1.2 ± 0.30</td>
<td>1.2 ± 0.30</td>
<td>1.2 ± 0.30</td>
<td>1.2 ± 0.30</td>
<td>1.2 ± 0.30</td>
</tr>
</tbody>
</table>

* Significant difference from 10°C (P<0.05).
** Significant difference from 10°C (P<0.00).
Fig. 3. Changes in opercular rates of carp during anesthesia and recovery with FA-100 at 10°C. (I) Reappearance of opercular movement; (II) Partial recovery of equilibrium with recovery of swimming motion; (III) Total recovery of equilibrium; (IV) Reappearance of avoidance swimming motion; (V) Total behavioral recovery. Each value represents the mean ± standard error of 3 to 5 fish.

Fig. 4. Changes in opercular rates of carp during anesthesia and recovery with FA-100 at 20°C. (I) Reappearance of opercular movement; (II) Partial recovery of equilibrium with recovery of swimming motion; (III) Total recovery of equilibrium; (IV) Reappearance of avoidance swimming motion; (V) Total behavioral recovery. Each value represents the mean ± standard error of 3 to 5 fish.

12.1 min at 100 ppm in 20°C. In recovery process, increasing the dosage of FA-100 increased the mean time required for each recovery stage except for Stage V at 20°C. Two fish exposed to 25 ppm of FA-100 at 10°C recovered to Stage I immediately after transfer to fresh water. Three fish exposed to 25 ppm of FA-100 at 20°C recovered to Stage I immediately after transfer to fresh water. Mean times required for total recovery (Stage
Table 5. Effects of anesthetic concentration and water temperature on times required to induce each anesthetic stage and for each recovery stage from anesthesia with RABONAL in the carp

<table>
<thead>
<tr>
<th>Water temperature (°C)</th>
<th>Concentration of RABONAL (ppm)</th>
<th>Induction time (min) to</th>
<th>Recovery time (min) to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage 1</td>
<td>Stage 2</td>
<td>Stage 3</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>6.9±1.6(5) a)</td>
<td>6.5±1.3(5)</td>
<td>11.3±2.7(5)</td>
</tr>
<tr>
<td>300</td>
<td>3.9±1.0(5)</td>
<td>4.8±1.0(5)</td>
<td>8.5±2.5(5)</td>
</tr>
<tr>
<td>20</td>
<td>3.9±0.6(5)</td>
<td>6.4±1.0(5)</td>
<td>12.3±3.4(5)</td>
</tr>
<tr>
<td>300</td>
<td>1.7±0.3(5)*</td>
<td>3.1±0.9(5)</td>
<td>4.4±1.2(5)</td>
</tr>
</tbody>
</table>

* Significant difference from 10°C (p<0.05).

* Mean±S.E. with number of fish in parenthesis.

Table 5 shows the effects of RABONAL concentration and water temperature on times required to induce each anesthetic stage and for recovery stage. Increasing the dosage of RABONAL (200 ppm) decreased the mean times required to induce each anesthetic stage. The opercular rates increased during recovery process. The opercular rates of fish exposed to 100 ppm of FA-100 were significantly (P<0.05) less in 20°C than in 10°C. Mean times required to reach Stage I and Stage II and III in fish exposed to FA-100 were significantly (P<0.05) less in 10°C than in 20°C. The opercular rates of fish exposed to 100 ppm of FA-100 were significantly (P<0.05) less in 20°C than in 10°C. Mean times required to reach Stage I and Stage II and III in fish exposed to FA-100 were significantly (P<0.05) less in 10°C than in 20°C. The opercular rates of fish exposed to 100 ppm of FA-100 were significantly (P<0.05) less in 20°C than in 10°C.
on fish during induction of anesthesia. In recovery process, increasing the dosage of RABONAL increased the mean times required for each recovery stage. Mean times required for total recovery (Stage V) were 76.4 min at 200 ppm and 83.0 min at 300 ppm in 10°C, and 50.2 min at 200 ppm and 70.0 min at 300 ppm in 20°C. One fish exposed to 200 ppm of RABONAL at 10°C recovered to Stage I immediately after trans-
fer to fresh water. Excitable reflexes to external stimuli still remained on fish after recovery to normal swimmings (Stage V).

Mean times required to induce each anesthetic stage in fish exposed to 300 ppm of RABONAL were less in 20°C of water temperature than in 10°C. The differences in mean times taking to reach Stage I anesthesia in fish exposed to 300 ppm of RABONAL were significant (P<0.05) between 10 and 20°C. Mean times required for each recovery stage were less in 20°C of water temperature than in 10°C except for Stage I recovery at 200 ppm.

Fig. 5 and 6 show the changes in opercular rates of fish during anesthesia and recovery with RABONAL at 10 and 20°C, respectively. There were trend to increase in opercular rates during induction of Stage I anesthesia. The opercular rates decreased during anesthesia. During recovery process, the opercular rates increased and returned to normal. The opercular rates of fish acclimated at 20°C were greater than those at 10°C.

No fish died during the experiment.

DISCUSSION

MS-222 is a close analogue of local anesthetic benzocaine. FA-100 was commonly used in dentistry as an anodyne [5]. RABONAL is a central nervous system depressant and its main site of action is on the cerebral cortex with secondary effects on mid-brain and brain stem core [6]. Stages of anesthesia and recovery of adult carp determined in this study were basically similar to the classification of the behavioral changes in the fish described by McFarland [13] and Kikuchi et al. [11]. However, this study did not discriminated between stage I-1 (light sedation) and stage I-2 (deep sedation) described by them. The recovery process was prescribed as the reversal of anesthetic process. Therefore, this study classified the anesthetic process in 5 stages and the recovery process in 5 stages. FA-100 (25 to 100 ppm) and RABONAL (200 to 300 ppm) shortened the time required to induce each anesthetic stage and delayed recovery with dose depended manner. Higher dosage of MS-222 (50 to 200 ppm) also hastened induction of anesthesia. However, there were no difference in recovery times between the used concentrations of MS-222, and the recovery was quicker than those observed in the case with other agents. These results indicate that the criterion of anesthesia and recovery determined in this study is useful for analyzing the effect of anesthetic in adult carp, and reveals the quick recovery of carp when it is anesthetized with MS-222.

It has been reported that changes in respiratory movements of fish by anesthesia are closely related with the depth of anesthesia [13, 20]. FA-100 and RABONAL caused the decrease in respiratory rates. The decrease in respiratory rates caused by them would be based on the inhibition of respiratory center in the medulla oblongata in relation to the depress of central nervous system. On the contrary, respiratory rates of carp exposed to MS-222 increased during anesthesia, and decreased during recovery and returned to normal. This result agrees with the other report [15] that MS-222 causes the increase in respiratory rates with a simultaneous increase in heart rates of the tench (Tinca tinca, L.). The mechanism of the increase in respiratory rates caused by MS-222 is not clear. But, it was suggested that some connection exists between cardiac and respiratory control system within the brain [15]. These differences in respiratory movements of carp between MS-222 and FA-100 anesthesia may exert different influences on blood-gas acid-base balance during anesthesia and recovery.

MS-222 is the most excellent of three anesthetics on rapid induction and recovery. It has been shown that the major entry and the excretion of anesthetics are through the gills, and emphasized that the penetration of anes-
thetic into gills depends on degree of ionization and the lipid solubility of the anesthetic [3, 10]. Rapid induction and recovery may be due to relatively high lipid solubility of the free base of MS-222. In addition, the movement of anesthetic across gills is presumably regulated by factors such as branchial ventilation, perfusion and effective exchange area [7, 8]. The quick recovery observed when carp was anesthetized with MS-222 might be in part the result of stimulated respiration.

As judged from anesthetic depth and the stability in recovery, it seems that 100–200 ppm MS-222 and 50–100 ppm FA-100 provide good anesthetic condition for surgical operations, and 50 ppm MS-222 and 25 ppm FA-100 provide good sedation on adult carp. Barbiturates such as amobarbital, secobarbital and pentobarbital were used in a solution for fish anesthesia [14]. In that report, the induction and recovery periods were very long. As RABONAL is an ultra-short acting barbiturate agent, it was used for examining if the anesthetic induction and recovery would be rapid in carp, and if it would be an effective anesthetic in carp as well as in mammals. But, carp exposed to RABONAL showed the excitible reflex to external stimuli and required long times (about 1 hour) for total recovery. Consequently, it seems that RABONAL can not be clinically used as an effective anesthetic in carp. As the recovery times from RABONAL anesthesia are very long, RABONAL may be of value in use for long duration experiment.

Results of the present study clearly demonstrate that the high water temperature augments the anesthetic effects of MS-222, FA-100 and RABONAL, and facilitate the recovery. These findings were in agreement with those of previous reports in Fundulus [13], rainbow trout, common carp, and fathead minnows [22], and medaka [2], which were anesthetized with MS-222 or FA-100. The results of a number of thermal stress experiments have demonstrated that increases in gill ventilation and cardiac rates occur as acclimation temperature are increased over a short time span [4, 16, 23]. Furthermore, in the present study, opercular rates at high temperature (20°C) were greater than those at low temperature (10°C). As the gills in fish are the main route of entry and the excretion of anesthetics [10], increasing gill ventilation and cardiac rates at high temperature would increase the gill permeability of anesthetic, and result in increasing the efficacy of anesthetic. Recovery from anesthesia would be rapid because of the high metabolic rates at high temperature.

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REFERENCES


要 約
トリカインメタノールフォネイト、オイゲノールおよびチオベンタールナトリウムによる鰤 (Cyprinus carpio) の麻酔と覚醒：日笠喜朗・高瀬勝明・小笠原俊美1・小笠原俊彦（北里大学臨床薬学部薬外科学教室, 家畜病院）—鰤におけるトリカインメタノールフォネイト (MS-222), オイゲノール (FA-100) およびチオベンタールナトリウム (RABONAL) の麻酔効果と覚醒について検討した。麻酔は薬浴法で行ない、覚醒および覚醒中の鰤の行動と呼吸数の変化、さらに麻酔と覚醒効果に対する水温 (10 および 20℃) の影響を調べた。FA-100 (25–100 ppm) および RABONAL (200 および 300 ppm) 濃度の上昇は、各麻酔ステージへの導入を速め、覚醒を遅延させた。高濃度の MS-222 (50–200 ppm) は麻酔の導入を速めたが、覚醒時間には濃度間に著変はみられず、他の剤と比較、速い覚醒を示した。FA-100 および RABONAL 麻酔により呼吸数は減少した。一方、MS-222 麻酔では、呼吸数は麻酔中増加し、覚醒中減少し、正常数に回復した。水温の上昇は、それぞれの麻酔薬による麻酔の導入と覚醒を共有に遅めた。