

モリネート暴露時に生ずるコイの血液凝固機能障害

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Hemostatic Disorder in Common Carp Induced by Exposure to the Herbicide Molinate*¹

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Hemostatic disorders in common carp induced by exposure to the herbicide molinate were investigated. Fish were exposed to molinate at 0.50 ppm for 9 days in Experiment 1, and at 0.32 ppm for 13 days in Experiment 2. Blood was collected from all specimens at various intervals, and activated partial thromboplastin times (APTT) and prothrombin times (PT) were determined. A prothrombin correction test was applied to the fish exposed to molinate, and the effect of menadione dimethylpyrimidinol bisulfite (MPB) in preventing the prolongation of clotting time was examined. The hemostatic disorders induced by exposing fish to molinate are summarized as follows: 1) Prolongations were observed in both APTT and PT after 6-7 days. Thereafter, the prolongation of PT became more pronounced than that of APTT. 2) The prolongation of PT was corrected by the addition of stored serum, suggesting that depleted coagulation factors exist in the serum. 3) Menadione was highly effective in preventing the prolongation of clotting times. These facts suggest that the hemostatic disorders resulted from a depletion of vitamin K-dependent factors.

In addition to severe hemorrhaging,¹⁾ prolongations of bleeding and clotting times have been reported in molinate-induced anemia in common carp.²⁾ Furthermore, it has been demonstrated that menadione is highly effective in preventing this anemia.^{2,3)} These findings suggest that the hemostatic disorders should play an important role in the occurrence of severe hemorrhaging.

The present study was undertaken to elucidate 1) whether the disordered hemostasis involves the intrinsic system, the extrinsic system, or both; 2) which group of coagulation factors, serum factors, or barium sulphate-adsorbed factors, are depleted; and 3) whether menadione dimethylpyrimidinol bisulfite (MPB) is effective in preventing the prolongation of clotting times.

Materials and Methods

Experiment

Two experiments were conducted in this study. In each, activated partial thromboplastin times (APTT) and prothrombin times (PT) were measured during the exposure of fish to molinate. Prothrombin correction testing was performed in Experiment 1, and the effect of MPB in preventing the prolongation of clotting time was examined in Experiment 2.

Experiment 1 Two groups of common carp *Cyprinus carpio*, each including 3 fish weighing about 483 g, were kept in two glass tanks. One group of fish was exposed to a test solution of molinate at a concentration of 0.50 ppm, and the other group served as a control, being exposed to acetone alone at a concentration of 50 ppm (Table 1).

Clotting times were determined for each fish at the beginning, 3, 6, and 9 days after the start of the experiment. In each measurement, 1.5 ml of blood was drawn from the caudal vessel into a plastic syringe containing 0.17 ml of 3.13% trisodium citrate solution, and a plasma sample was

Table 1. Composition of test solutions

Experiment 1			
Group	Molinate (ppm)	MPB* (ppb)	Acetone (ppm)
1	0.50	0.0	50
2	0.00	0.0	50
Experiment 2			
Group	Molinate (ppm)	MPB* (ppb)	Acetone (ppm)
1	0.32	0.0	50
2	0.32	32.4	50
3	0.00	0.0	50

* MPB: menadione dimethylpyrimidinol bisulfite.

*¹ Studies on anemia of fish-XII.

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obtained by centrifuging the blood at 3,000 rpm for 5 min. Procedures for the determination of APTT and PT were described in the previous papers.^{4,5} A prothrombin correction test was applied to the group of fish exposed to molinate after 10 days.

The experimental apparatus consisted of a glass tank (90×45×45 cm) and an auxiliary tank (45×45×47 cm), through which 140 l of test solution was circulated. Seventy liters of test solution was renewed every day during the period of the experiment. The water temperature was maintained at $25 \pm 1^\circ\text{C}$. To prepare the test solution of molinate, 0.5 ml of stock solution was dissolved in 10 l of water. The stock solution was prepared by dissolving 1,000 mg of molinate in 100 ml of acetone. To prepare the test solution for the control group, 0.5 ml of acetone was dissolved in 10 l of water. The fish were fed daily with a commercial pellet food.

Experiment 2 Three groups of common carp, each including 3 fish weighing about 480 g,

were kept in three glass tanks. The first group of fish was exposed to molinate at a concentration of 0.32 ppm. The second group was exposed to molinate and MPB at concentrations of 0.32 ppm and 32.4 ppb, respectively. The third group served as a control and was exposed to acetone alone at a concentration of 50 ppm (Table 1). Test solutions for the first and third groups were prepared in the same manner as in Experiment 1, except for the concentration of molinate. To prepare the test solution for the second group, 0.5 ml of molinate stock solution and 1.0 ml of MPB stock solution were dissolved in 10 l of water. The molinate stock solution was prepared by dissolving 640 mg of molinate in 100 ml of acetone, and the MPB stock solution was prepared by dissolving 32.4 mg of MPB in 100 ml of distilled water. The experimental apparatus was the same as in Experiment 1. Seventy liters of test solution was renewed every day, and the water temperature was maintained at $25 \pm 1^\circ\text{C}$. The fish were fed a commercial pellet food.

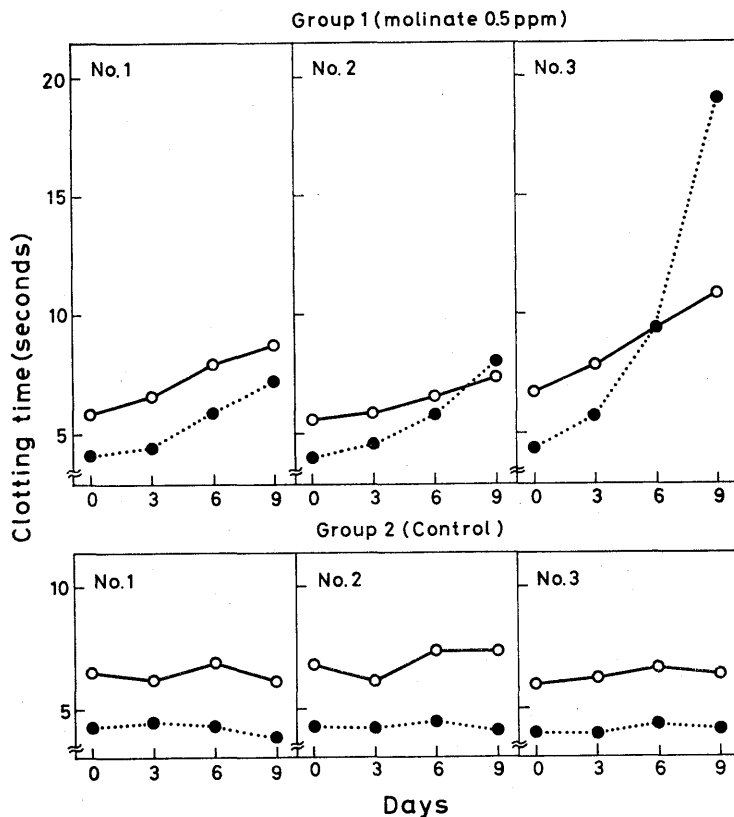


Fig. 1. Changes in APTT and PT during exposure to molinate (Experiment 1). Clotting times are plotted individually in each group. Each group includes three specimens.

APTT: ○—○; PT: ●-----●.

APTT and PT were determined for all fish at the beginning, 7, 10, and 13 days after the start of the experiment.

Prothrombin Correction Test

Two correction reagents, *i.e.*, stored serum and barium sulphate-adsorbed plasma, were prepared by the following procedures.

Stored serum: Equal volumes of sera collected from 3 healthy carp were pooled and kept at 4°C for 2 days until used.

Barium sulphate-adsorbed plasma: Citrated plasma was prepared on the day the test was performed. Equal volumes of citrated plasma collected from 3 healthy carp were pooled. Barium

Table 2. Prothrombin correction test in Experiment 1

Plasma	PT(s) before mixing	PT(s) after mixing with	
		BaSO ₄ -adsorbed plasma	Stored serum
No. 1	8.9	9.2	6.9
No. 2	11.3	11.8	8.1
No. 3	34.6	37.9	8.7

sulphate was added to the plasma (400 mg/ml) and the mixture was stirred with a plastic rod for 15 min at 25°C. After centrifugation at 3,000 rpm for 5 min, the supernatant was stored at 4°C.

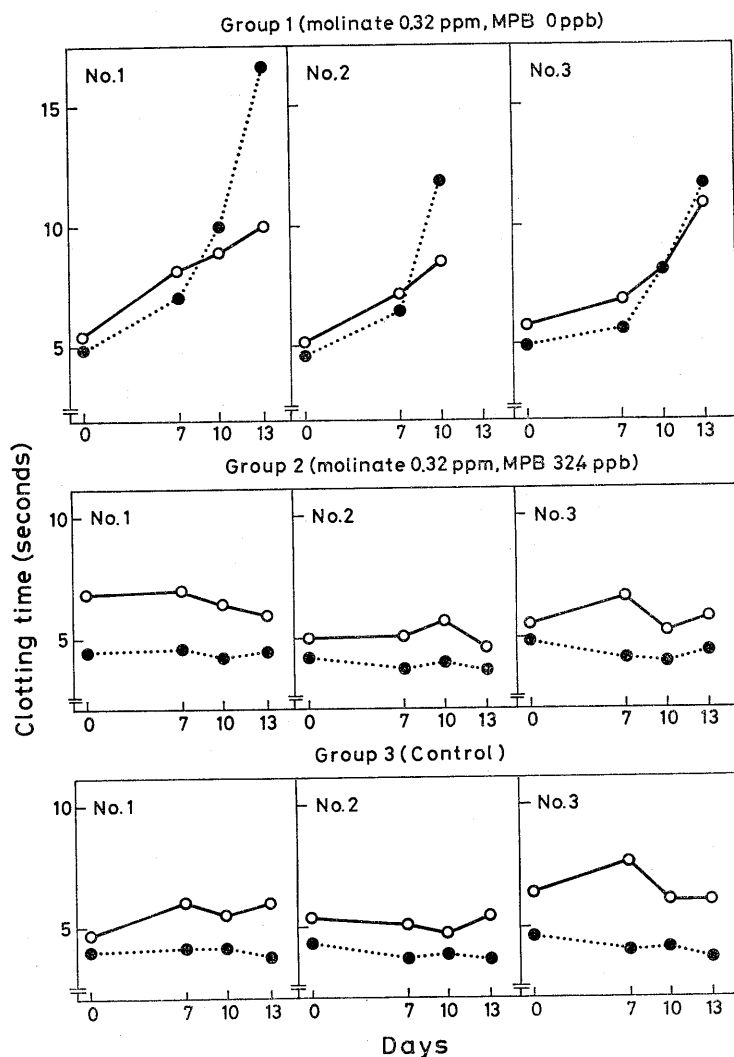


Fig. 2. Changes in APTT and PT during exposure to molinate (Experiment 2). Clotting times are plotted individually in each group. Fish No. 2 in Group 1 died after 11 days. APTT: ○—○; PT: ●-----●.

Test plasma samples were collected from 3 fish exposed to molinate by drawing 4.5 ml of blood into 0.5 ml of 3.13% trisodium citrate solution and centrifuging it at 3,000 rpm for 5 min. Two plasma mixtures were prepared from each test plasma: (1) 9 volumes of test plasma +1 volume of stored serum, and (2) 9 volumes of test plasma +1 volume of barium sulphate-adsorbed plasma. The PT was determined for each plasma mixture and original test plasma.

Results

Experiment 1

Prolongations of APTT and PT were observed in all fish exposed to molinate, while no noticeable change was found in the controls (Fig. 1). In the control group, APTT and PT maintained levels of 6.0–7.4 s and 3.8–4.4 s, respectively, during the course of the experiment. In the group of fish exposed to molinate, APTT and PT increased to 7.0–10.8 s and 7.3–18.9 s, respectively, after 9 days. The increase in PT was almost equal to that of APTT during the first 3 days, but subsequently it became greater than that of APTT.

Prothrombin correction testing revealed that the prolonged clotting time was shortened by the addition of stored serum, whereas no improvement was observed by the addition of barium sulphate-adsorbed plasma (Table 2). This indicates that serum factors were depleted from the blood during the exposure to molinate.

Experiment 2

Prolongations of APTT and PT were reconfirmed in the first group of fish exposed to molinate (Fig. 2). Changes in the clotting times revealed similar trends observed in Experiment 1. In this group, one fish that showed a rapid prolongation of PT died after 11 days. In the second group of fish exposed to molinate and MPB, no recognizable change was observed in clotting times, suggesting that MPB was highly effective in preventing the prolongation of clotting time.

Discussion

The hemostatic disorders observed in common carp during the exposure of fish to molinate are summarized as follows:

1) Prolongations were observed in both APTT and PT, and the increment of PT gradually became larger than that of APTT.

2) The prolongation of PT was corrected by the addition of stored serum, whereas no improvement was observed by the addition of barium sulphate-adsorbed plasma.

3) Menadione was highly effective in preventing the prolongation of clotting time.

In the mammalian coagulation mechanism, the sequence of reactions in the intrinsic and extrinsic pathways finally activates factor X to Xa, and these two pathways are joined to a common pathway in which prothrombin conversion and clot formation are involved. The existence of a similar fundamental coagulation system is accepted in bony fishes, whereas the intrinsic pathway is lack or incomplete in the lamprey⁶⁾ and elasmobranchs.^{6,7)} The remarkable prolongations of PT observed in the present study suggest that the intrinsic and extrinsic pathways acted independently and that the disorder in the extrinsic pathway was more pronounced than in the intrinsic one.

Prothrombin correction testing demonstrated that the depleted factors during exposure to molinate are supplemented from serum and not from barium sulphate-adsorbed plasma. In addition, menadione was highly effective in preventing the prolongation of clotting time. Although knowledge of coagulation factors participating in the coagulation mechanism is limited in fishes, except that fibrinogen and prothrombin are involved in a common pathway,^{6,8,9)} it may safely be assumed that depleted factors during exposure to molinate belong to serum factors and also to vitamin K-dependent coagulation factors.

The prolongation of clotting time in vitamin K deficiency has been observed in brook trout,^{10,11)} On the other hand, no prolongation of clotting time has been reported in channel catfish fingerlings that were fed synthetic diets without supplemental vitamin K.¹²⁾ These findings indicate that the requirement for vitamin K is different among fish species. Although a vitamin K requirement has not been reported in common carp, it is reasonable to consider that vitamin K is essential for the maintenance of hemostasis in common carp blood and that a disturbance of vitamin K function or an exhaustion of vitamin K consumption exaggerated hemorrhaging during exposure to molinate.

References

- 1) H. Kawatsu: *Nippon Suisan Gakkaishi*, **43**, 905–912 (1977).

- 2) T. Ochiai and S. S. Kubota: *Bull. Facul. Fish. Mie Univ.*, **5**, 129–153 (1978).
- 3) H. Kawatsu and T. Ikeda: *Nippon Suisan Gakkaishi*, **54**, 1731–1736 (1988).
- 4) H. Kawatsu and M. Sato: *Nippon Suisan Gakkaishi*, **53**, 1533–1536 (1987).
- 5) H. Kawatsu and K. Kondo: *Nippon Suisan Gakkaishi*, **55**, 183 (1989).
- 6) R. F. Doolittle and D. M. Surgenor: *Am. J. Physiol.*, **203**, 964–970 (1962).
- 7) J. Lewis: *Comp. Biochem. Physiol.*, **42A**, 233–240 (1972).
- 8) R. F. Doolittle, J. L. Oncley, and D. M. Surgenor: *J. Biol. Chem.*, **237**, 3123–3127 (1962).
- 9) H. Perrier, J. Delcroix, C. Perrier, and J. Gras: *Comp. Biochem. Physiol.*, **49B**, 679–685 (1974).
- 10) H. A. Poston: *Prog. Fish-Cult.*, **26**, 50–63 (1964).
- 11) W. Steffens: *Int. Revue ges. Hydrobiol.*, **59**, 255–282 (1974).
- 12) T. Murai and J. W. Andrews: *Nippon Suisan Gakkaishi*, **43**, 785–794 (1977).