Effect of Oral Administration of Warfarin on Blood Coagulation in the Common Carp

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The anticoagulant effect of potassium warfarin and the antagonistic effect of vitamin K₃ against warfarin were examined using common carp. Three different dosages of warfarin with and without supplementary vitamin K₃ were orally administered to common carp for 10–14 days, and prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured at intervals of 3–7 days. At dosages of 2.0 and 1.0 mg/kg/day of warfarin, prolongation of PT and APTT was observed. Supplementation of vitamin K₃ was effective in preventing the prolongation of PT at a dosage of 1.0 mg of warfarin, but it was not effective at 2.0 mg. On the other hand, supplementation of vitamin K₃ could not prevent prolongation of APTT with any warfarin dosage used, indicating that the antagonistic effect of vitamin K₃ against warfarin is limited to PT.

In previous papers we demonstrated that the blood coagulation mechanism is disordered in common carp exposed to molinate and that vitamin K is effective in preventing this effect.^{1,2)} Vitamin K is necessary for the biosynthesis of several essential coagulation factors in the hemostatic system, and the action of vitamin K is antagonized by various anticoagulants.³⁾ These facts led us to suppose that the pharmacological effect of molinate is similar to that of anticoagulants. A number of studies have been reported on anticoagulation using coumarin drugs in mammals, whereas very little research has been performed using fish. Murai and Andrews⁴⁾ reported that administration of pyvalyl (pyvalyl-1,3-indandione) greatly prolonged prothrombin time in the catfish *Ictalurus punctatus*, whereas administration of dicumarol, orally or intravenously, showed no effect on the prothrombin time.

The present paper describes the effect of oral administration of potassium warfarin on the hemostatic system in common carp.

Materials and Methods

Animals

One-year-old common carp *Cyprinus carpio* were used in this study. Prior to testing, the fish were held in the experimental apparatus for more than 1 week and fed on a commercial pellet

food.

Materials

Potassium warfarin was supplied by Eisai Co., Ltd., Tokyo. Vitamin K₃ was purchased from Nacalai Tesque, Inc., Kyoto. Activated partial thromboplastin reagent, "Actin", was from Baxter Dade Diagnostic, Inc., Aguada, Puerto Rico. Cellulose powder (>200 mesh) was from Toyo Roshi Co., Ltd., Tokyo. All other reagents were of analytical grade.

Experiment

Three experiments were conducted in this study. In each, different dosages of potassium warfarin were administered to fish: 2.0 mg/kg/day in Experiment 1, 1.0 mg/kg/day in Experiment 2, and 0.5 mg/kg/day in Experiment 3.

Experiment 1: Four groups of common carp, each including 2 fish (average 478 g), were administered with four different test diets. The first group was given a diet containing warfarin alone, the 2nd and 3rd groups were given diets containing warfarin and vitamin K₃, and the last group, which served as a control, was given a diet without supplementary warfarin and vitamin K₃. The dosage of warfarin was 2.0 mg/kg/day, and the dosages of vitamin K₃ were 0.1 mg/kg/day in the 2nd group and 1.0 mg/kg/day in the 3rd group. To prepare the test diets, 1 part of cellulose powder

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containing vitamin K₃ and 9 parts of commercial powdered food were mixed with 60 parts of warfarin solution. Then, the pasty test diet was orally administered using a catheter on the ration of 1.2 ml per 100 g body weight per day (1.2 ml of pasty diet contained 0.2 g of solid constituents). Potassium warfarin was dissolved in distilled water immediately before preparation of the diet (20 mg/100 ml). To prepare the cellulose powder containing vitamin K₃, K₃ was dissolved in ethanol and mixed with cellulose powder (0.5 mg/g and 5.0 mg/g), then dried in a decompression desiccator. The test diet was given once in the morning every day during the period of the experiment. After administration of test diet, a commercial pellet food was given as supplemental food every day.

Activated partial thromboplastin time (APTT) and prothrombin time (PT) were determined for all fish at the beginning of the experiment and after 3, 7, and 10 days.

The experimental apparatus consisted of a glass tank (90×45×45 cm) and an auxiliary tank (45×45×47 cm), through which 160 l of water was circulated. Approximately half the volume of water was renewed every day. The water temperature was maintained at 25±1°C.

Experiment 2: Four groups of fish, each including 3 fish (average 412 g), were administered with four different test diets. The compositions of test diets were same as in Experiment 1, except for the amount of warfarin. In Experiment 2, 10 mg of potassium warfarin was dissolved in 100 ml of distilled water. Clotting times were measured for all fish at the beginning of the experiment, and after 7 and 14 days. The test diet was not administered on the day before measurement of clotting times. The experimental apparatus was the same as in Experiment 1, and the water temperature was maintained at 25±1°C.

Experiment 3: Three groups of fish, each including 2 or 3 fish (average 475 g), were administered with three different test diets. The first group was given a diet containing warfarin alone, the 2nd group was given a diet containing warfarin and vitamin K₃ and the last group was given a diet without supplementary warfarin and vitamin K₃. The dosages were warfarin 0.5 mg/kg/day and vitamin K₃ 0.1 mg/kg/day. Clotting times were measured for all fish at the beginning, and after 7 and 14 days. The test diet was not administered on the day before measurement of clotting times. The experimental apparatus was the same as in Experiment 1, and the water tem-

perature was also maintained at 25±1°C.

Procedures for the Measurement of Clotting Time

In each measurement, 1.5 ml of blood was drawn from a caudal vessel into a plastic syringe containing 0.17 ml of 3.13% trisodium citrate solution, and a plasma sample was obtained by centrifuging the blood at 3,000 rpm for 5 min.

For the determination of APTT, a commercially available reagent, "Actin", was used. One-tenth ml of "Actin" solution was pipetted into a polystyrene tube (10×72 mm) and kept in a 37°C water bath for 1 min. Then 0.1 ml of the test plasma was added and kept for 2 min. Finally 0.1 ml of M/40 calcium chloride solution was added and stopwatch was started. The clotting time was taken as the time when a fibrin clot formed.

For the determination of PT, the common carp brain extract was used. Procedures for the preparation of brain extract and for the measurement of PT were described in a previous paper.⁵⁾

Results

Results from three experiments are collectively illustrated in Figs. 1 and 2, in which each symbol with vertical bar represents mean±SD.

Experiment 1: Prolongation of PT and APTT was observed in all groups of fish given potassium warfarin. At a dosage of 2.0 mg/kg/day of warfarin, supplementation of vitamin K₃ did not prevent prolongation of PT and APTT. In the control group, no noticeable change was observed during the course of the experiment.

Experiment 2: In contrast to the results of Experiment 1, prolongation of PT was observed only in the first group of fish, which received potassium warfarin alone (Fig. 1). In the second and third groups of fish which received warfarin and vitamin K₃, no prolongation was observed in PT. These results indicate that supplementation of 0.1 mg/kg/day of vitamin K₃ was sufficient to prevent the prolongation of PT at a dosage of 1.0 mg/kg/day of warfarin. On the other hand, it is noteworthy that supplementation of vitamin K₃ could not prevent the prolongation of APTT (Fig. 2).

Experiment 3: The prolongation of PT in the first and second groups was very small (Fig. 1). Although the difference in PT between these two groups was within 1.0 s, prolongation in the first group was slightly larger than that in the second group, suggesting that supplementation of vitamin K₃ was effective in preventing the prolongation

Fig. 1. Dose-response relationships between PT and oral administration of warfarin with and without supplementation of vitamin K_3 . Standard deviations are shown by vertical bars, some of which are covered by the size of the symbol used. ●—●: warfarin alone; ■-----■: warfarin + vitamin K_3 (0.1 mg/kg/day); ▲-----▲: warfarin + vitamin K_3 (1.0 mg/kg/day); ○-----○: control.

Fig. 2. Dose-response relationships between APTT and oral administration of warfarin with and without supplementation of vitamin K_3 . Standard deviations are shown by vertical bars. ●—●: warfarin alone; ■-----■: warfarin + vitamin K_3 (0.1 mg/kg/day); ▲-----▲: warfarin + vitamin K_3 (1.0 mg/kg/day); ○-----○: control.

of PT. On the other hand, APTT showed a slight prolongation, which was a similar trend observed in Experiment 2.

Discussion

The anticoagulant effect of warfarin was observed in both PT and APTT at dosages of 2.0 and 1.0 mg/kg/day, but was not observed at 0.5 mg. These results indicate that susceptibility to warfarin in common carp is lower than that in rats. Hagan and Radomski⁹⁾ reported that PT was prolonged to 66–76 s in rats fed 1.0 mg/kg of warfarin daily for 2 days (24–25 s in controls).

The antagonistic effect of vitamin K₃ with respect to warfarin was only observed in PT; it was not observed in APTT. The antagonistic effect of vitamin K₃ was dependent on the dosage of warfarin, i.e., vitamin K₃ was effective in preventing the prolongation of PT at a dosage of 1.0 mg/kg/day warfarin, but was not effective at 2.0 mg/kg/day.

In common carp exposed to molinate, we observed that vitamin K₃ (menadione dimethylpyrimidinol bisulfite) was effective in preventing the prolongation of both PT and APTT.¹⁾ In the oral administration of warfarin, it is difficult to explain why vitamin K₃ was not effective in preventing the prolongation of APTT, although it was effective on PT. In Experiment 2, two groups of fish that received warfarin and vitamin K₃

showed normal PT and prolonged APTT values. Although vitamin K dependent factors have not been individually confirmed in fish, it may be supposed that certain K dependent factors involved in the intrinsic pathway were affected more severely than those involved in the extrinsic pathway. Viewed from another angle, certain coagulation factors other than K dependent factors might possibly be affected by warfarin. These questions remain for future study.

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