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ABSORBABILITY OF FACTOR VIII OR IX CONCENTRATES PREPARATION USING BEAGLES

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ABSTRACT

Facoor VIII or IX concentrates preparation was modified with activated methoxy polyethylene glycol (PEG), and administered orally to beagles to investigate absorbability from the intestines.[1] Factor VIII or IX concentrates preparation modified with PEG was absorbed from the intestines. Changes of platelet counts and appearanse of FDP was presumed to be an additional phenomenon.[2] Factor VIII or IX concentrates preparation with no modification were not absorbed from the intestines.

INTRODUCTION

Hemophilia A (Factor VIII deficiency) and hemophlia B (Factor IX deficiency) are hereditary haemorrhagic diathesis with prolonged coagulation time, and in Japan are observed in one per 22,000 males with sex-linked recessive type.[1]

Von Willebrand's disease is observed in one per 35,000 persons with prolonged bleeding time with autosomal dominant or recessive type, and is caused by disordered platelet aggregation and Factor VIII complex anomaly.[2]

Haemorrhagic manifestations of hemophilia A or B and von Willebrand's disease differ from each other; the former show subcutaneous, intramuscular, intestinal and joint bleeding, and the latter shows nasal bleeding and petechia.

For the stoppage of the haemorrhage, infusion of Factor VIII or IX concentrates preparation is the preferred treatment and is performed 2 or 3 times weekly in a severe case. Home infusion therapy for prophylaxis enables the patients to function normally. If oral administration is effective in increasing the Factor VIII or IX level in the plasma,

the danger of bleeding will be greatly decreased, and will benefit the patients.

We reported on the oral administration of Factor VIII or IX concectrates with a preparation treated by liposomes to patients who volunteered and to beagles and found an increase of coagulation factors in the plasma.[3,4]

In this work, we administered Factor VIII or IX concentrates preparation modified with activated PEG in order to investigate how to increase the Factor VIII or IX level in the plasma of beagles.

Materials and Methods

(1) Coagulation factor concentrates preparation:

Factor VIII concentrates preparation; Koäte (Factor VIII 500 units/vial, Cutter Co.) was used. Factor IX concectrates preparftion; Konyne (Factor IX 400 units/vial, Cutter Co.), which contained Factor II,VII, IX and X, was used.

(2) Activated polyethylene glycol (PEG):

Methoxy polyethylene glycol ($CH_3O(CH_2O)_3H$) (mean M.W. 4,700) was activated by cyanuric chloride using Abuchowski's methods.[5]

(3) Coagulation factor modified with the activated PEG:

One vial of Factor VIII or IX concentrates preparation was dissolved in 20 ml of 0.05 M phosphate buffer (pH 7.2) and added to aprotinin solution (5,000 units/ml, Trasylol, Bayer Co.) in the ratio of 400 units of the coagulation factor activity to 0.83 units of the aprotinin. 100 mg of the activated PEG was added to the mixture, and stored at 0°C for 3 hours. After these procedures, the mixtures were dialysed against 0.05 M phosphate buffer (pH 7.2) for one hour, and saline solution for 30 minutes, and thereafter kept in a frozen state.

(4) Administration method:

The materials from (3) were dissolved into 20 ml of distilled water, and given with 50 ml of milk to two beagles weighing 10kg each.

(5) Collection of the specimen:

Whole blood was mixed with 3.8% sodium citrate solution at 9 to 1, and centrifuged at 3,000 rpm for 15 min to obtain the plasma.

(6) Methodology of coagulation-fibrinolytic analysis:

Platelet counts, aPTT, PT, fibrinogen and coagulation factor activity by one stage method were determined. FDP was assayed by the latex aggregation method using dog's FDP anti-serum. These assays were performed at 0, 1, 2, 4, 6, 8 and 24 hours after the oral administration.

(7) Calculation of absorbability:

100 x assayed increased activity of coagulation factor (%)/expected increased activity of coagulation factor (%). Expected increased activity calculated as follows; Factor VIII (%) = 2 x administered Factor VIII concentrates (units) / body weight (kg), Factor IX (%) = 1 x administered Factor IX concentrates (units) / body weight (kg)

RESULTS

(1) Changes of coagulation with oral administration of Factor VIII concentrates preparation modified with PEG:

The modified Factor VIII (500 units) was given orally to the beagles. APTT and PT were shortened after the administration, and the level of Factor VIII was increased for eight hours, as shown in figures 1 and 2. The absorbability rate calculated was 92%.

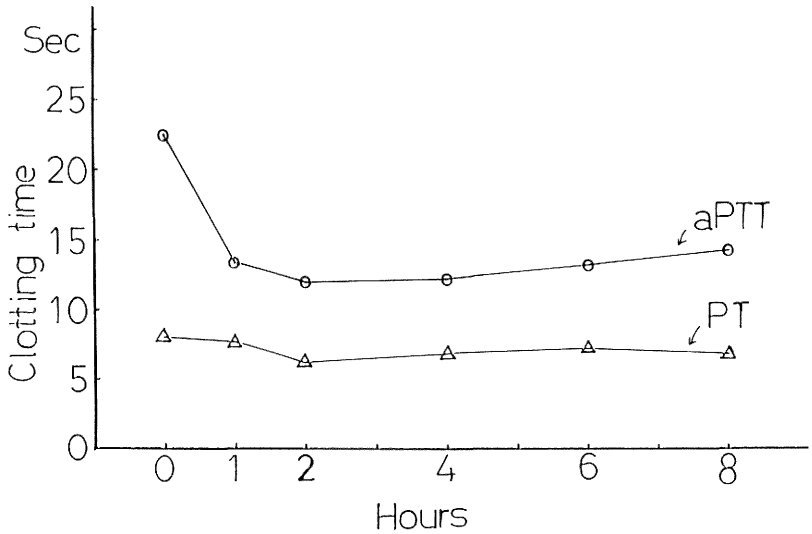

Fig. 1. Changes in aPTT and PT by oral administration of PEG modified Factor VIII preparation.

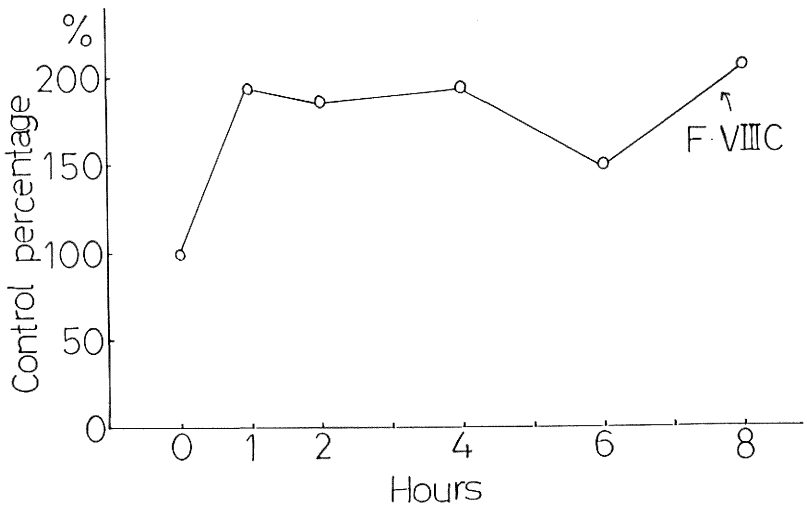

Fig. 2. Increase in factor VIIIC by oral administration of PEG modified factor VIII preparation.

Fibrinogen did not change markedly, but FDP and platelet counts increased as shown in figure 3.

(2) Changes of coagulation with oral administration of Factor IX concentrates preparation modified with PEG:

Factor IX concentrates preparation (Factor IX: 400 units) was administered orally to the beagles. APTT and PT were shortened and Factor IX increased up to 133% two

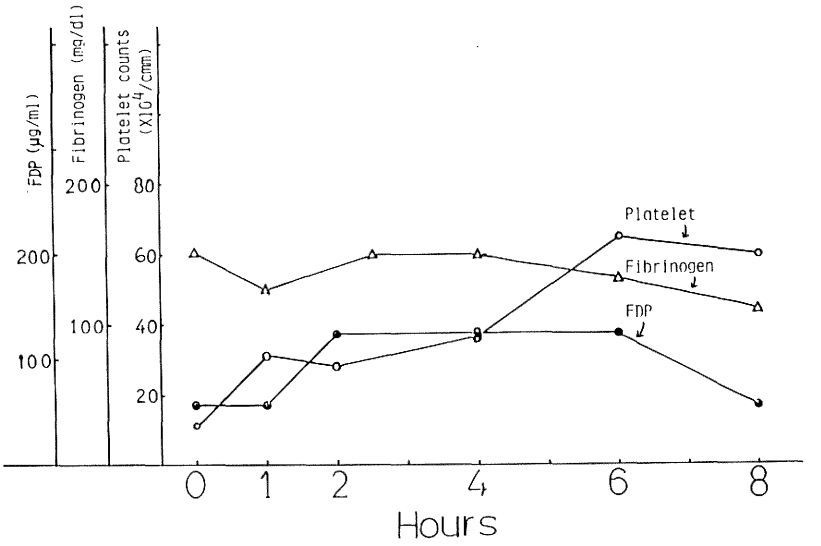

Fig. 3. Changes of platelets, fibrinogen and FDP by oral administration of PEG modified Factor VIII preparation.

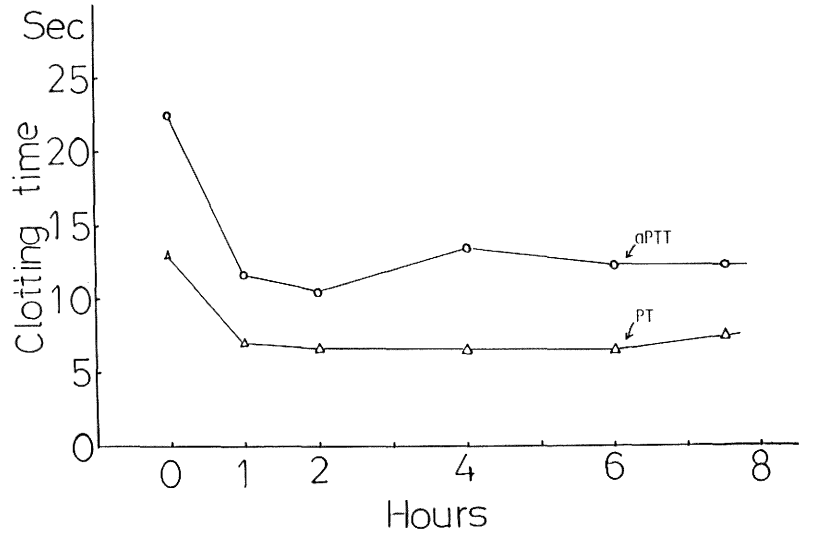

Fig. 4. Changes of aPTT and PT by oral administration of PEG modified Factor IX preparation.

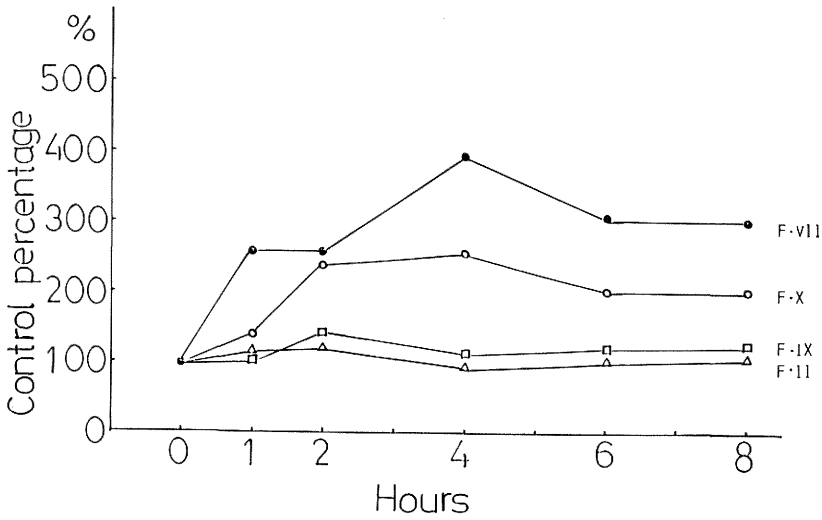

Fig. 5. Changes of Factors II, VII, IX and X by oral administration of PEG modified Factor IX preparation.

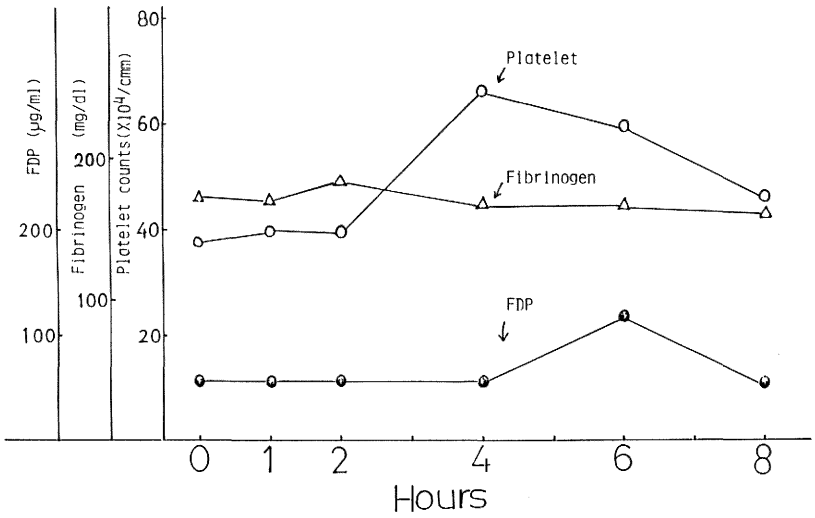

Fig. 6. Changes of platelet counts, fibrinogen and FDP by oral administration of PEG modified Factor IX preparation.

hours after administration. Factor VII and X increased makedly, as shown in figures 4 and 5. The absorbability of modified Factor IX concentrates was 83%.

FDP increased at six hours after administration, but fibrinogen did not. Platelet increased at four and six hours after administration, as shown in figure 6.

(3) Changes of coagulation with oral administration of Factor VIII or IX concentrates preparation with no modification:

Factor VIII concentrates (500 units) or Factor IX concentrates (Factor IX: 400 units) preparation was administered orally with milk. No significant changes of the coagulation system were observed in either case.

DISCUSSION

Oral treatment for haemorrhage in the cases of haemophilia is strongly desired.[3)4)] Our previous report gave methods[6)] for using aprotinin to prevent damages by proteases in the gastrointestinal tracts. Our experiments showed that Factor VIII and IX concentrates did not work well, being only from 4 to 15% absorbably effective. Therefore, it is necessary to devise a new mode for the preparation to increase the absorption rate, or we must devise cheaper coagulation factor concentrates by bio-technological methods.

In this report, we used activated PEG instead of liposome, expecting that it would have the efficacy to prevent the occurrence of antibodies against coagulation factor proteins, and to prolong the life-span of the coagulation factors that we had expected.

Increase of platelet counts was observed after oral administration, and this we could not account for. A possible reason for it might be the occurrence of an additional phenomena after absorption of PEG in the blood.

The increase of FDP could be caused by the hypercoagulation induced by the coagulation factors, especially in the case of Factor IX concentrates preparation, which contained active Factor X and thrombin.[7,8)] When a large amount of Factor IX concentrates preparation is administered with PEG, much care should be taken to prevent the occurrence of thrombosis.

To stop haemorrhage in a hemophilia case, it is necessary to infuse the coagulation factor properly. Nowadays in Japan the patients can infuse themselves at home, and stop haemorrhage quickly. However, if oral administration can attain good absorbability results in man, patients can take prophylactics orally.

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