Novel Drug Delivery System Using Autologous Fibrin Glue
—Release Properties of Anti-cancer Drugs—

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To clarify the release properties of anti-cancer drugs from fibrin glue, a study was performed using several anti-cancer drugs with remarkably different physical properties. Concentrated fibrinogen, fibronectin, and coagulation factor XIII (F-XIII), thrombin, and CaCl₂.¹ These components are mainly produced from human plasma and have high affinity for human tissues. Fibrin glue coagulates through a physiological hemostatic mechanism and adheres to bleeding tissue.² As fibrin glue is gradually lysed to fragments by plasmin of fibrinolytic enzyme, it does not last long. These properties indicate that fibrin glue could be useful as a base for controlled release products. Several types of fibrin glue products are commercially available,³ but all have some risk for infection by thermosetable pathogenicities,⁴ and for allergic reaction to subtype components of the glue.⁵–⁹ To prevent these adverse effects, cryoprecipitate (Cryo) produced from a patient's own autologous plasma is recommended for use as the fibrin glue.⁴,¹⁰ The purpose of this study is to clarify the release properties of anti-cancer drugs in fibrin glue produced from a volunteer’s plasma and the physico-chemical factor(s) affecting these properties.

MATERIALS AND METHODS

Materials The thrombin, aprotinin, and CaCl₂ solutions used were Thrombin-Yoshitomi (10000 U/vial) (Yoshitomi Pharm. Ind., Oska, Japan), Trasyrol (10000 U/ml) (Bayer Yakuhin Ltd., Osaka), and 2% CaCl₂-Otsuka (Otsuka Pharm. Ind., Tokyo, Japan), respectively. Fluorouracil, tegafur, mitomycin C, doxorubicin, etoposide, and enocitabine were used as anti-cancer model drugs and were obtained from commercial sources.

Human plasma of healthy volunteers, excluded by alanine aminotransferase (ALT) test, were obtained from the Yamaguchi Red Cross Blood Center (Yamaguchi, Japan).

The assay kit used for fibrin degradation products (FDP) was FDP reagent (ND) (International Reagent Co., Kobe, Japan). All chemicals were used obtained from commercial sources and were of analytical reagent grade.

Preparation of Cryo Preparation procedures were described in our previous paper in detail.¹² Briefly, the human plasma was frozen in a −20℃ freezer (MDF-U536, Sanyo Medica Systems Co., Osaka). It was shaken during this period by a horizontal shaker (NR-3, Taitec Co., Saitama, Japan), at a speed of 70 cycles/min. The plasma was then defrosted in a cold water bath (4℃), and centrifuged at 3000rpm for 15 min at 0–4℃. The supernatant was removed, and the precipitate was collected as Cryo. Cryo is a viscous liquid and contains 43±7 mg/ml of fibrinogen, 6.1±1.9 mg/ml of fibronectin, 15.9±4.8 units/ml of F-XIII, 2.2±0.2 units/ml of plasminogen, and a small amount of other plasma components.¹³

Lysing Test of Fibrin Glue Containing Anti-cancer Drugs The Cryo (100 µl) was mixed with each anti-cancer drug (40 µl) and aprotinin (400 U/ml) (10 µl) in sterilized microcentrifuge tubes (Robins Scientific, California), then a mixture of thrombin (100 U/ml) and CaCl₂ (0.4%) was added while agitating the solution.¹⁴ This mixture was incubated for 10 min at 37℃, and then centrifuged at 12000 rpm for 5 min. Thus generated, the fibrin glue was washed with 500 µl of PBS, and incubated in 1 ml of PBS containing plasminogen (100 µg/ml) and urokinase (100 U/ml) at 37℃. The medium (30 µl) was then sampled several times after centrifugation (12000 rpm, 2 min). Dispensing and sampling procedures were performed in a clean booth.

Analytical Methods Anti-cancer drug concentrations were measured in each sample using HPLC (LC-10A, Shimadzu Co., Kyoto, Japan). The conditions used were as follows; column: STR ODS-II 15 cm×4.6 mm (Shinwa Chemical Ind., Ltd., Kyoto), mobile phase: H₂O/CH₃OH=95/5 for fluorouracil, H₂O/CH₃OH=65/35 for tegafur and mitomycin C, 50 mM CH₃COONH₄/C₂H₅OH=65/35 for etoposide and doxorubicin, H₂O/CH₃OH=2/98 for enocitabine, flow rate: 1.2 ml/min, wavelength: 230 nm, injection volume: 10 µl, temperature: 37℃. Protein and FDP concentrations in each sample were measured using the Lowry method and latex agglutination method, respectively.

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Drug Hydrophobicity Hydrophobicities of each drug were represented by their retention times on the hydrophobic HPLC column (STR ODS-II 15 cm×4.6 mm). The conditions used were as follows; mobile phase: H₂O/CH₃OH = 5:95, flow rate: 1.2 ml/min, wavelength: 230 nm, injection volume: 10 µl, temperature: 37 °C.

Protein Binding The percentage binding of each drug to protein was cited from Drug Interview Forms published by the drug manufacturers.

Measurement of Adhesive Strength Adhesive strength of the fibrin glue was measured as follows: two ground flat pieces of glass (prepared from Micro slide glass of S-2215, Matsunami Glass Ind., Ltd., Osaka) of 4 cm², bound to cubic wood with chemical adhesive, were prepared. Ten microliters of Cryo was applied to one piece and 10 µl of the mixture of thrombin and anti-cancer drug to the other. The two applied surfaces were pressed together at 50 g/cm² for 30 s. After 10 min, the bonded pieces of glass were fixed in a tension measuring device (SV-55, Imada Manufact. Co., Ltd., Toyohashi) and the tensile strength of each fibrin glue was monitored in the vertical direction for adhesive surface. The adhesive strength was determined as the maximum strength.

Initial Release Rate The rate of initial release (Rir) was calculated as follows: Rir[%/2 h]=(drug concentration in supernatant 2 h after fibrin glue with aprotinin in incubation) [µg/ml/2 h] (maximum drug concentration in supernatant after fibrin glue without aprotinin in incubation) [µg/ml] X 100 [%].

Statistical Analysis Each value was expressed as the mean±S.D. of 4 experiments. Comparisons between the presence and absence of aprotinin were made using Student's unpaired t-test and one-way analysis of variance (ANOVA) followed by Scheffe's test with StatView J4.02 for Macintosh, and differences were considered to be statistically significant when p<0.05.

RESULTS AND DISCUSSION

Lysing Property of Fibrin Glue In the case of the fibrin glue without aprotinin, the protein concentration in the supernatant increased rapidly and reached a steady state level after 2—4 h (Fig.1A). While with aprotinin, the protein concentration increased immediately after the incubation was started and then gradually increased. Without aprotinin, the FDP concentration in the supernatant increased quickly and the fibrin glue disappeared after 2—4 h, similar to the time course of the protein concentration (Fig.1B). With aprotinin, the glue was only slightly hemolyzed and about half remained after 7 d. Therefore, the initial time course of the FDP concentration differed markedly from that of the protein concentration detected by the Lowry method. These results indicate that aprotinin is important for controlling the speed with which fibrin glue is lysed. The Lowry method is a quantitative method of analysis based on the reduction by tyrosine, tryptophan or cysteine contained in protein. It is well known that the protein concentration in sample determined by the Lowry method is remarkably different to that obtained using standard. So, it is difficult to compare directly the protein content obtained by the Lowry method with that obtained by specific methods of analysis such as FDP. Therefore, the difference in the protein and FDP concentration profile in the presence of aprotinin may be due to a water-soluble protein such as the albumin contained in Cryo. Namely, water-soluble protein is detected by the Lowry method but not by the FDP method. It is well known that fibrin glue is composed of a mesh structure. The mesh size of the net may be larger than the molecular size of albumin. So, it is necessary to choose a drug with affinity for the fibrin glue in order to prepare a sustained-release product.

Release Properties of Anti-cancer Drugs Fluorouracil mixed in the glue was released quickly and reached a steady state level after 1—2 h regardless of the presence or absence of aprotinin (Fig. 2). The same results were obtained at all concentrations of drug added to the glue. Taguafur and mitomycin C acted the same as fluorouracil. So, these anti-cancer drugs appear not to interact with the glue. The level of etoposide in the supernatant increased rapidly, reached a maximum after 6 h, and then rapidly decreased, in the absence of aprotinin. However, in the presence of aprotinin, these concentrations increased gradually, peaked after 24 h, and then gradually decreased regardless of the anti-cancer drug concentra-
tions (Fig. 3). The reason for the decrease in the etoposide concentration in the supernatant may be degradation and crystallization of the drug. Doxorubicin acted the same as etoposide. Enoticabine was gradually released from the glue with aprotinin during the experiments, although it was more quickly released from the glue without aprotinin (Fig. 4). These results indicate that the release properties of anti-cancer drugs in fibrin glues differ remarkably.

**Physical and Release Properties of Anti-cancer Drugs**

To clarify the factor(s) regulating the release of the anti-cancer drugs in the glue, a study was made of the relationship between the physical properties of anti-cancer drugs and their Rir from the glue with aprotinin. For each drug, the Rir from the glue with aprotinin correlated well with hydrophobicity, but not with molecular weight or protein binding (Fig. 5). Moreover, neither acidity nor basicity was observed to relate to the rate of release. These results indicate that to achieve a sustained release using fibrin glue, one should use the more lipophilic anti-cancer drugs.

**Effect of Anti-cancer Drug on the Adhesive Strength of Fibrin Glue**

Generally, there are two methods for the clinical application of fibrin glue containing anti-cancer drug. Namely, direct injection into the carcinoma tissue, and application to the cut section of remaining tissue after the carcinoma tissue has been surgically removed, to prevent the recurrence of the cancer. In the latter case, the adhesive as well as the drug release property of the glue is important. To clarify the effect of each anti-cancer drug on the adhesive strength of fibrin glue, glue containing various concentrations of drug with thrombin were applied to ground flat glass and the strength of the glue was monitored. As shown in Fig. 6, the adhesive strength of control was similar to that of each glue containing anti-cancer drug. These results indicate that the adhesive strength of fibrin glue was not affected by anticancer drug under our experimental conditions.

In this paper, we described the release properties of anti-cancer drugs in fibrin glue produced from volunteer's plasma and the physico-chemical factor(s) affecting these properties. Fibrin glue without aprotinin was quickly hemolyzed, but that with aprotinin was hardly hemolyzed and more than half remained after 7 d. The release rate of each drug was correlated well with their hydrophobicity. Thus, to establish a sus-

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**Fig. 3.** Release Properties of Etoposide from Fibrin Glue  
A: without aprotinin, B: with aprotinin; ●; 200 µg, ▲; 100 µg, ■; 50 µg. Mean±S.D. (n=6), *; p<0.01 (with vs. without aprotinin).

**Fig. 4.** Release Properties of Enoticabine from Fibrin Glue  
A: without aprotinin, B: with aprotinin; ●; 200 µg, ▲; 150 µg, ■; 100 µg. Mean±S.D. (n=4), all points: p<0.01 (with vs. without aprotinin).

**Fig. 5.** Relationship between the Physical and Release Properties of Anti-cancer Drugs  
●, fluorouracil; ▲, tegafur; ▲, mitomycin; ○, doxorubicin; ●, etoposide; ▲, enoticabine. A: Rir vs. retention time (logY=1.20 - 1.77 logX, r=0.994). B: Rir vs. protein binding (logY=2.27 - 0.49 logX, r=0.682). C: Rir vs. molecular weight (logY=4.48 - 1.14 logX, r=0.661).

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Fig. 6. Effect of Anti-cancer Drug on the Adhesive Strength of Fibrin Glue

Mean±S.D. (n=4).

REFERENCES


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