

Original Article

Nafamostat Mesilate Reduces Blood Cell Adhesion to Cardiopulmonary Bypass Circuits: An In-Vitro Study

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ABSTRACT

Nafamostat mesilate (FUT-175) is a protease inhibitor, working as an inactivator of coagulation, fibrinolysis and platelet aggregation. Although FUT-175 directly blocks contact factors in coagulation, it also may decrease activation of humoral cascade systems when used in cardiopulmonary bypass circuits. We performed an in vitro study using fresh human blood in the following cardiopulmonary bypass circuits: standard circuit (C), biosurfaced circuit (B) and standard circuit containing FUT-175 (F). Each circuit was primed with 500 ml of electrolyte solution and 500 ml of fresh blood. Cardiopulmonary bypass was performed using a roller pump for four hours in two sets of each circuit configuration. Platelet factors (platelet count and *beta*-thromboglobulin), coagulation factors (thrombin-antithrombin III complex and fibrinopeptide A), fibrinolysis factors (*alpha* 2-plasmin inhibitor complex and *alpha* 2-plasmin inhibitor), complement factors (C3a, C4a), free hemoglobin, and granulocyte elastase were measured at the beginning and end of the study. Hemocytograms were measured concurrently. The FUT-175 group showed significantly lower levels of the measured indices than the biosurfaced group in thrombin-antithrombin III complex (7.4 ± 2.1 vs. 54.9 ± 38.1 ng/ml), fibrinopeptide A (7.2 ± 2.0 vs. 20.2 ± 14.6 ng/ml), *beta*-thromboglobulin (1940 ± 250 vs. 2438 ± 314 ng/ml) and free hemoglobin (25.2 ± 14.3 vs. 73.8 ± 18.4 mg/dl). There were no significant differences between Group F and Group B in platelet count, C3a, C4a and granulocyte elastase, although these indices were significantly lower in Groups F and B when compared to Group C. Therefore, FUT-175 appears to reduce blood-foreign surface reaction and diminishes cellular adhesion to the inner surface of the cardiopulmonary bypass circuit.

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INTRODUCTION

During cardiac surgery, blood is exposed to the artificial surfaces of extracorporeal circuits. This exposure results in activation of coagulation, fibrinolysis and platelets (1-3). Excessive fibrinolysis and/or transient impairment of platelet function may be the cause of abnormal bleeding tendencies after cardiac surgery (4-7). Biosurfaces like heparin-coated surfaces in extracorporeal circuits have been used to reduce activation of humoral cascade systems. Nafamostat mesilate^a (6-amino-2-naphthyl p-guanidinobenzoate dimethanesulfonate) is a synthetic serine protease inhibitor. FUT-175 works as an inactivator of coagulation, fibrinolysis and platelet aggregation, having potent inhibitory activity with respect to thrombin, activated coagulation factors (XIIa, Xa), kallikrein, plasmin and complement factors (C3a, C4a) (8,9). Inhibitory activity of coagulation factor XIIa reduces the contact phase in the coagulation system. Therefore, FUT-175 may decrease activation of humoral cascade systems when used as a biosurface material in cardiopulmonary bypass (CPB) circuits. We performed an in vitro study of CPB with fresh human blood to estimate the inactivation capacity of FUT-175.

MATERIALS AND METHODS

An in vitro study of CPB was performed on three different extracorporeal circuits: control (C), biosurfaced (B) and FUT-175 (F). The CPB circuits of Groups C and F contained a non-heparin coated membrane oxygenator^b, arterial line filter^c, cardiotomy reservoir^c and polyvinyl chloride tubing. Group B used the same arterial line filter and cardiotomy reservoir, but included a heparin-coated membrane oxygenator^b and Bionate^d tubing, a new biosurfaced material recently developed in Japan.

Each CPB circuit was primed with 500 ml of electrolyte solution and 500 ml of fresh human blood. Ten milligrams of FUT-175, which was dissolved in a 5% glucose solution (1 mg/ml) was added to the priming solution of Group F. Heparin (1000 IU) and sodium bicarbonate (1.4 g) were injected prior to initiating CPB. The fresh human blood was reconstituted with CaCl₂ (0.55 g). Additional heparin was administered when the activated clotting time (ACT) fell below 400 seconds. CPB was performed using a rotary pump^e at a flow rate of 3.0 L/min for four hours. Blood temperature was maintained at approximately 28°C with a heat exchanger.

Activation of platelets, coagulation and fibrinolysis were estimated with blood markers. For estimation of platelet activation, the platelet count and blood concentration of *beta*-thromboglobulin, which is a factor released from platelet *alpha*

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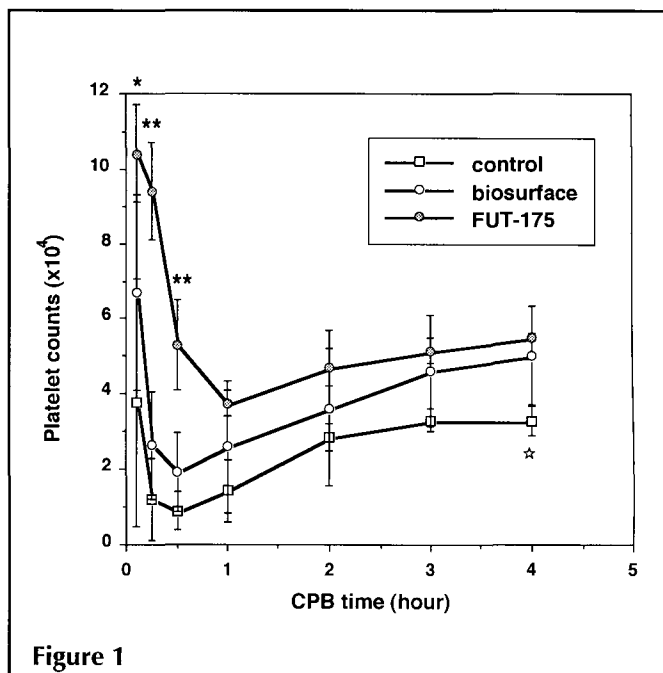


Figure 1

granules, were measured. As markers for coagulation, blood concentrations of thrombin-antithrombin III complex (TAT) and fibrinopeptide A (FPA) were measured with enzyme-linked immunosorbent assay (ELISA). TAT indicates thrombin production. FPA is also a marker for thrombin production since it is released from fibrinogen, which is activated by thrombin. As a marker for fibrinolysis, *alpha* 2-plasmin inhibitor complex (PIC) concentration, which indicates plasmin production, was measured with enzyme immunoassay (EIA). Blood concentrations of complement (C3a, C4a), free hemoglobin (free Hgb) and granulocyte elastase were also measured. Blood sampling was done at the beginning and end of the study for all measurements. Hemocytograms were measured consecutively at the 5th, 15th and 30th minute of CPB as well as at the end of the 1st, 2nd, 3rd and 4th hour since initiation of CPB. Activated clotting time was measured by Hemochron^f according to the same sampling schedule. Plasma was separated from the heparinized blood samples by centrifugation for 10 minutes within 30 minutes of sample collection, then stored at -80°C until analysis. Duplicate measurements were performed on each sample. Differences between groups were evaluated by the non-paired t-test.

Results were expressed as mean \pm standard deviation in the text and as mean \pm standard error in the figures.

RESULTS

ACTIVATED CLOTTING TIME

In Group C, the ACT was approximately 400 seconds during the study and additional heparin was given in some cases. On the other hand, the ACT was approximately 1000 seconds in Group B and more than 1500 seconds in Group F with no additional heparin required.

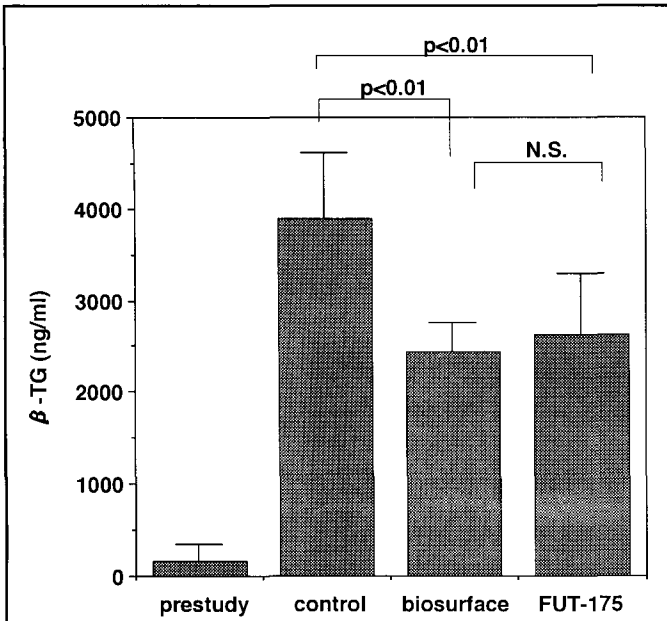


Figure 2

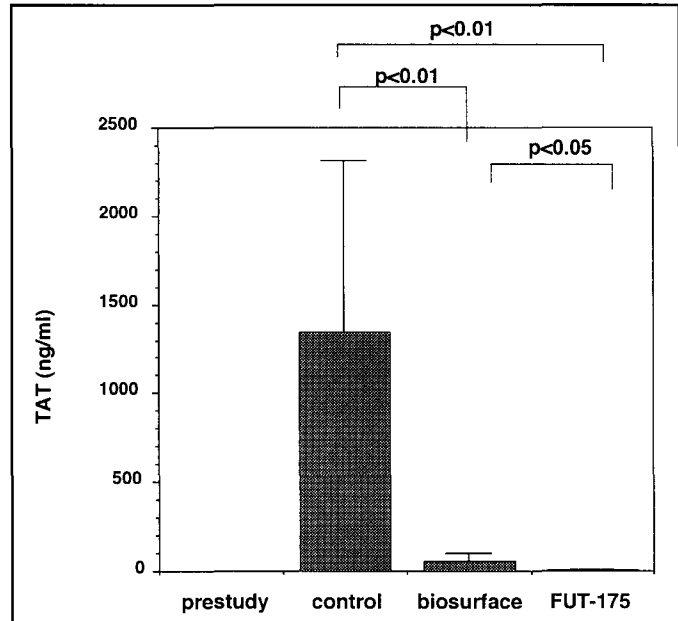


Figure 3

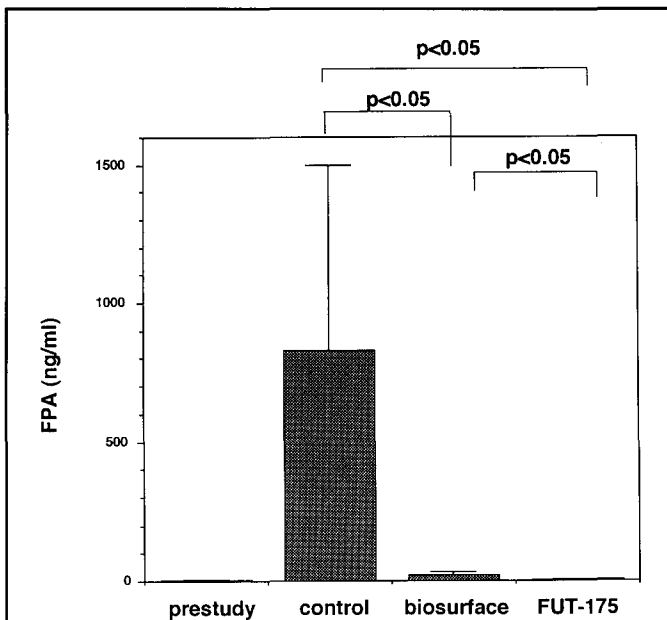


Figure 4

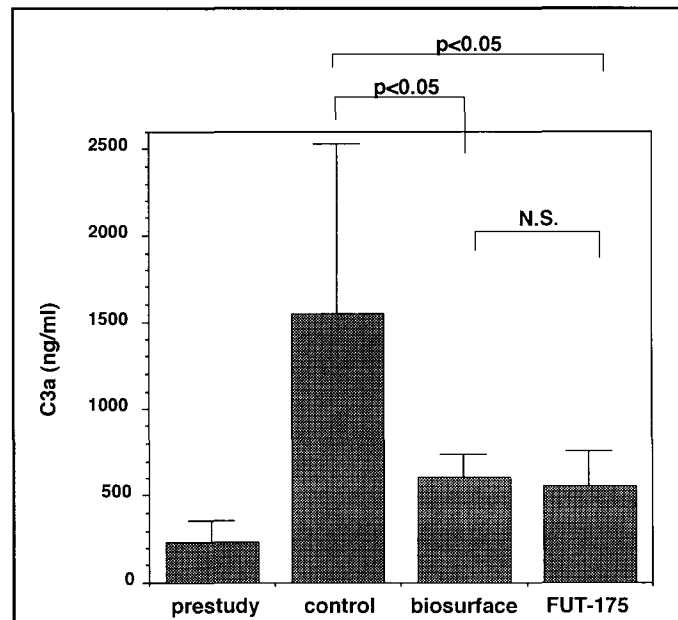


Figure 5

PLATELET FACTORS

Platelet count in Group C decreased rapidly after initiating CPB and remained at approximately 10,000/mm³ after 30 minutes. In Group B the platelet count decreased slowly after initiating CPB and remained at approximately 30,000/mm³. Platelet count decreased least in Group F, remaining around 50,000/mm³ during the study, which was higher than both Groups B and C (p<0.01) during the first 30 minutes of CPB (Figure 1).

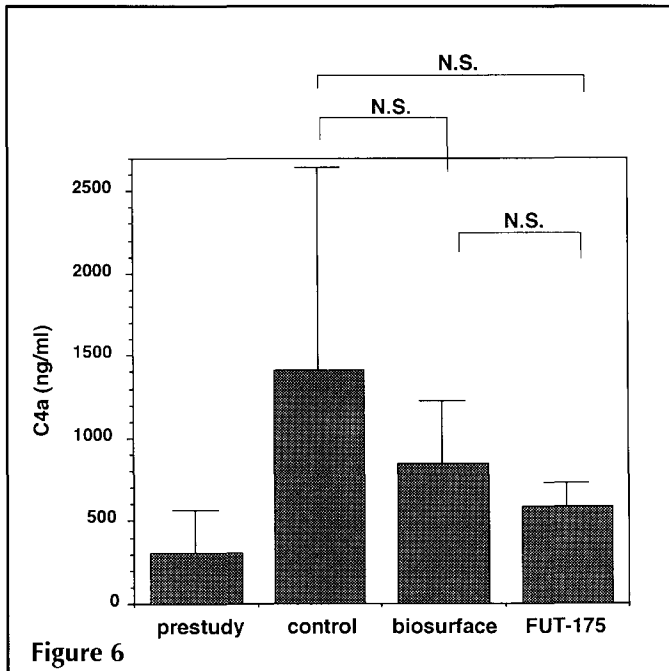
Beta-TG concentrations increased in all three groups during the study. However, the 20-fold increase in Group C was

significantly larger than Groups B and F. The increase in beta-TG was smaller in Group F than in Group B (p<0.05) (Figure 2).

COAGULATION FACTORS

TAT concentrations in Group C climbed dramatically, showing a 600-fold increase. Group B, however, increased 25-fold while Group F had very little change. There were significant differences between each group (Figure 3).

FPA concentrations also increased dramatically in Group C, with significant differences between Groups B and F. FPA



showed only a 3-fold increase in Group B and very little change in Group F (Figure 4).

FIBRINOLYSIS FACTORS

PIC concentrations did not increase significantly in any of the groups during the study and there was no significant difference between the groups. PIC concentrations decreased only in Group C, showing a significant difference when compared to Groups B and F.

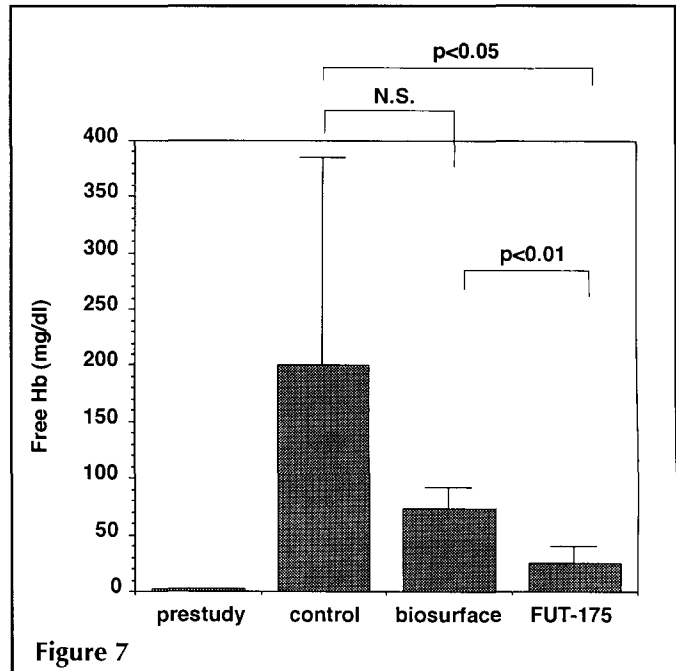
OTHER FACTORS

Blood concentrations of complement in Group C changed significantly during the study, showing a 6-fold and 4-fold increase in C3a and C4a, respectively. C3a concentrations in Group C were significantly higher than Groups B and F. However, there was no significant difference among groups in C4a concentrations (Figures 5 and 6).

Free Hgb concentration increased in all groups during the study. Group F showed a significantly lower concentration of free Hgb at the end of the study than Groups B or C (Figure 7). Blood concentration of granulocyte elastase showed an 8-fold increase in Group C, which was significantly different than the small rise in Groups B and F (Figure 8).

DISCUSSION

FUT-175 is a new synthetic protease-inhibiting agent which has potent inhibitory activity to thrombin, coagulation factors in active form (XIIa, Xa), kallikrein, plasmin, complement factor (C3a, C4a) and trypsin. Affinity of FUT-175 prevents activation of the coagulation system by inhibiting thrombin through creation of the thrombin-FUT-175 complex without



dependence on antithrombin III, and by inhibiting factors XIIa and Xa (11). As FUT-175 inhibits activation of factor XII, the contact phase in coagulation is blocked and activation of the humoral cascade systems is decreased. Therefore, FUT-175 can reduce blood/foreign surface reactions and diminish cellular adhesion to the inner surfaces of extracorporeal circuits.

FUT-175 undergoes hydrolysis in the blood and liver before undergoing glucuronic acid conjugation. Elimination of FUT-175 from the bloodstream is rapid, with a half-life of several minutes during normothermia. However, elimination rate was reduced with hypothermia. In a previous clinical study, FUT-175 concentration was approximately 2,000 ng/ml with hypothermia when infused continuously at a rate of 40 mg/hr (12). In the present study, FUT-175 was added to the priming volume at a concentration of 10 mg per 1000 ml. Its concentration was approximately 10,000 ng/ml (10^{-5} M) at the beginning of the study. Elimination of FUT-175 is considered negligible and its concentrations may be maintained during an in vitro study. Therefore, FUT-175 concentration in this study may be similar or a little higher than in actual clinical studies.

In the present study, activation of coagulation was inhibited most strongly in the group using FUT-175. Degranulation of platelets was also diminished most strongly by FUT-175 and platelet counts in the FUT-175 group were maintained higher than in the biosurfaced Group B. In the concentrations used in this study, FUT-175 works just as other biosurfaced materials and also inhibits activation of humoral cascade systems and prevents platelet aggregation as a serine protease inhibitor. On the other hand, there was no significant difference between groups with respect to fibrinolysis. However, FUT-175 suppressed fibrinolysis strongly in clinical studies (12). This discrepancy may be caused by the differences between the in vitro and in vivo

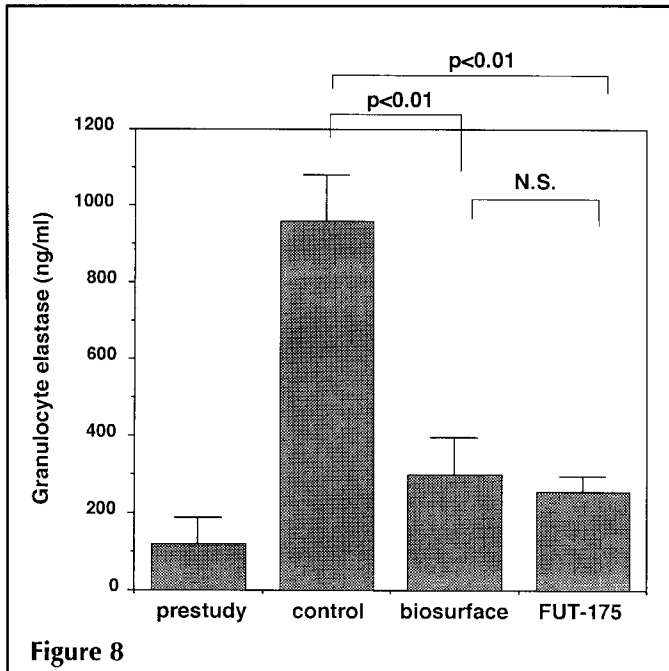


Figure 8

environments.

FUT-175 suppressed complement production of both C3a and C4a. This result indicates that FUT-175 inhibits both the classical and alternative pathways of complement activation. FUT-175 appeared to reduce hemolysis, showing lower free Hgb levels than did the other biosurfaced CPB circuit. Blood concentration of granulocyte elastase showed similar levels at the end of the study in both the FUT-175 and biosurfaced groups.

In conclusion, FUT-175 reduces platelet activation, coagulation, complement production, hemolysis and granulocyte activation. FUT-175 has been used frequently in hemodialysis as an anticoagulant, but its use in CPB is still experimental. Future investigation with this drug is required to confirm its safety in cardiac surgery.

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