PROTEASES II

Potential Role in Health and Disease

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FIBRINOLYSIS CAUSED BY CARDIO-PULMONARY BYPASS AND SHED MEDIASTINAL BLOOD RETRANSFUSION - IS IT OF CLINICAL RELEVANCE?

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INTRODUCTION

The importance of blood saving methods during cardiac surgery is well accepted. It is commonly known that, in spite of heparin treatment, systemic coagulation and fibrinolysis will be activated by cardio-pulmonary bypass (CPB). Whether this activation is of clinical relevance was many times a point of discussion (1). Similar changes were found in the shed mediastinal blood postoperatively (2), which was retransfused to reduce homologous blood requirement (3). It is generally accepted now, that this method leads to a reduction of homologous blood requirement in cardiac surgery (4), but some reservations were made because of the low quality of the chest tube blood (5).

The aim of our study was to investigate the degree of fibrinolysis in the shed blood and in patients' circulation, the influence of lysosomal enzymes of desintegrated or activated granulocytes upon fibrinolyses, and to answer the question, whether retransfusion of shed mediastinal blood causes clinically relevant changes in patients' hemostasis.

METHODS

In twenty adult patients undergoing cardiac surgery parameters of fibrinolysis were analysed at the following instances: 1. before operation, 2. after CPB (comparing the data obtained at these two instances the effect of CPB on fibrinolysis can be estimated), 3. in the intensive care unit before and 4. 20 minutes after retransfusion. Samples were taken from patients' circulation as well as from the chest tube drainage. The applied analytical methods for measurements of plasminogen, antiplasmin, D-dimer, early fibrin(ogen) degradation products (N-terminal Bß-fragments 1-42 and 15-42) and elastase from PMN granulocytes complexed with a_1-proteinase-inhibitor (E-a_1PI) are shown in Table 1. Retransfusion was only performed when at least 250 cc blood were collected and when it was indicated by the patient's circulatory state.

Table 1. Methods

plasminogen	-	chromogenic substrate S 2251 (Kabi)
antiplasmin	-	chromogenic substrate S 2251 (Kabi)
D-dimer	-	monoclonal antibodies (ELISA/MabCo)
N-terminal Bß-fragments	-	monoclonal antibodies (ELISA/NIH)
E-a_1PI	-	PMNE (ELISA/Merck)

For statistical analysis the paired t-test comparing the measurements before and after retransfusion was applied. A p-value $< 0,05$ was considered to be significant. Results are given as mean $\pm$ SD.

RESULTS

The mean amount of the first chest blood retransfusion was 325 $\pm$ 77 cc (ranging from 250 - 520 cc). The mean hemoglobin content of the chest drainage was 69 g/l, the mean hematocrit 21 % (ranging from 13 - 29 %), the protein content 40.1 $\pm$ 5.4 g/l.

Figure 1 shows the course of plasminogen and antiplasmin activity, the dotted line representing the value corrected for preoperative protein. When using this correction for hemodilution no statistically significant influence of CPB on these parameters was found. However, antiplasmin activity in the drainage fluid was significantly reduced. No difference of activity in the patients' blood could be demonstrated before and after retransfusion. The surprising fact that no reduction of plasminogen activity in the chest drainage was measured might be explained by the influence of fibrin and fibrin(ogen) split products on the measurement of plasminogen (6).

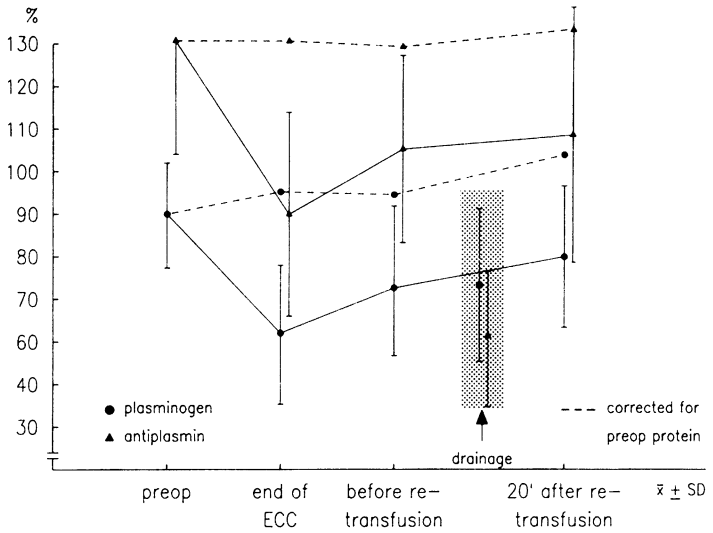

Fig. 1 Course of plasminogen and antiplasmin. The dotted line represents the value corrected for preoperative protein.

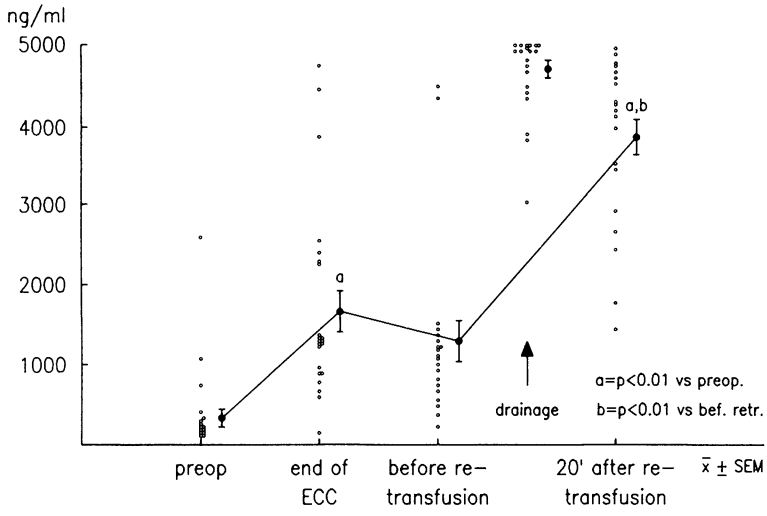

Fig. 2 Cross-linked fibrin fragments. Retransfusion of of shed blood caused a significant ($p < 0,01$) increase of the concentration in patients' circulation compared to values before retransfusion.

The cross-linked fibrin fragments (Figure 2) showed a wide variation after CPB. The concentration decreased after operation, but developed a tremendous increase up to 5,000 ng/cc in the drainage fluid. The retransfusion caused a significant increase of cross-linked fibrin fragments in patients' blood.

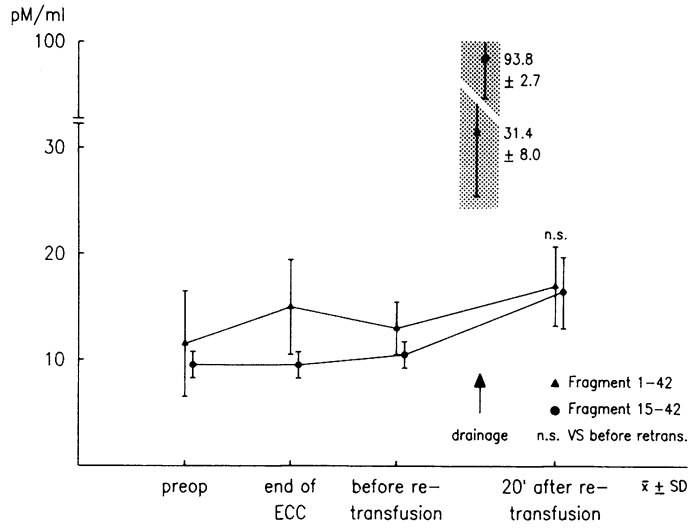

Fig. 3 Bß-related peptides. Note the interruption of the Y-axis. Despite the high concentration in the drainage blood there was - in contrast to the cross-linked fibrin fragments - no significant increase in patients' circulation after retransfusion.

The Bß-related peptides (Figure 3) showed a comparable course during CPB with an increase of the 1-42 fragments which was not significant. In the chest tube drainage a more pronounced increase of Bß 15-42 peptides was found. However, there were no differences of Bß-related peptides in patients' blood before and after retransfusion.

The PMN E-a_1PI complex (Figure 4) showed a tenfold increase in patients' blood after CPB, but there was an extraordinary increase in the drainage fluid with a mean concentration of 13,000 ng/cc - more than hundredfold the preoperative value. Retransfusion also caused a significant increase of the E-a_1PI complex in patients' blood. These values were significantly higher than those obtained prior to retransfusion.

No significant correlation between either blood loss or time lag between operation and retransfusion and fibrinolysis, represented by the antiplasmin activity, could be found in the shed blood. After retransfusion blood loss did not exceed the average of normal range.

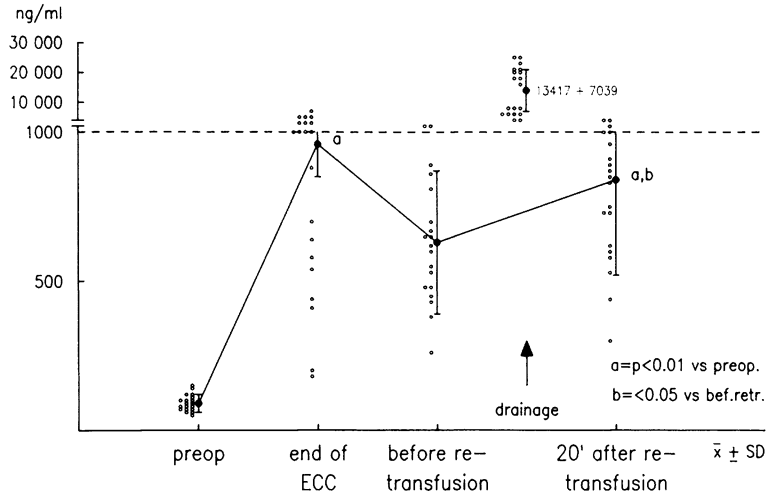

Fig. 4 PMN E-a_1PI complex. Note the interruption and different scale of the Y-axis. There was a tremendous increase in the shed blood. Retransfusion of shed blood caused a significantly increased level of the complex in patients' blood.

DISCUSSION

Shed mediastinal blood is not comparable to autologous or homologous (7) blood in regard to hemoglobin content, hematocrit or coagulation properties. However, in former investigations (8), comparing two groups of patients with and without retransfusion of shed mediastinal blood, no difference in the postoperative blood loss could be observed.

In the present study an activation of the fibrinolytic system in shed mediastinal blood was found. The magnitude of fibrinolytic activation in shed blood was comparable to that measured during CPB. This activation of the fibrinolytic system was indicated by comparable decreases of antiplasmin activity in patients' blood after CPB as well as in the shed blood. However, retransfusion of shed blood did not cause an additional decrease of antiplasmin in the patients' circulation.

Bß-related peptides are a most sensitive marker of the endogenous activation of the fibrinolytic system, which can reveal subclinical activation of fibrinolysis (9). A pronounced increase of fibrin(ogen) split products was found in the shed blood. After retransfusion only the D-dimers showed a significant increase in patients' blood, whereas the concentration of Bß-related peptides showed no increase. This can be explained by the lower molecular weight and therefore faster clearing rate of these peptides.

The constellation of increased fibrin(ogen) split products and stable antiplasmin activity after retransfusion can be interpreted as a waste effect of retransfusion. That means, the enhanced fibrinolytic or proteolytic activity within the shed blood is inhibited by the physiologic inhibitors. Some of these complexes and the split products obviously remain in the circulation after retransfusion without inducing fibrinolytic activity within the patient.

In this study the elevated levels of complexed elastase after CPB correspond to the results of other authors (10) indicating an activation or desintegration of PMN granulocytes due to CPB. The extraordinary increase of E-a_1PI in the shed blood may be explained by desintegration of granulocytes caused by mechanical alteration due to pericardial and/or pleural movement in the thorax and in the chest tubing.

Which conclusions can be drawn from the results of this study?

The fibrinolytic and unspecific proteolytic activity in the drainage fluid does not induce fibrinolytic activation after retransfusion. The increased level of fibrin(ogen) split products seems not to be clinically relevant, at least not with the amounts having been retransfused. It can be concluded that retransfusion of shed mediastinal blood is a save method of autologous volume substitution in the early postoperative period. However, it has to be taken into account that retransfusion of greater volumes of shed blood may cause a considerable impairment of coagulation and/or the fibrinolytic system. This deterioration might be induced by the infusion of fibrin(ogen) split products and/or the release of the content of desintegrated granulocytes. In this respect the possible role of pharmacological intervention by protease inhibition should be object of further investigations.

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