THROMBIN AND FIBRINOGEN EXTRACTED FROM PORCINE BLOOD USED AS A CLOTTING AGENT

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Background

Thrombin is responsible for the conversion of fibrinogen into fibrin thus forming a clot. Fibrinogen can be isolated from blood plasma and it is used in clinical haematology and veterinary therapeutics.

Huang et al.(1992), Divakaran(1982) prepared fibrin foam and haemostat from porcine blood and studied their effectiveness of clotting. It was found that the capability to clot the blood and plasma readily and firmly was widely applied in the control capillary bleeding, fibrin suturing of tissues and skin grafts. If fibrinogen and thrombin can be applied at the same time when the human or animals were injured and bled, the hemostatic action may be more effective.

Objectives

To extract thrombin and fibrinogen from porcine blood and added with calcium chloride to prepare a haemostat solution and investigate its effectiveness of clotting.

Methods

Porcine blood was collected from the local slaughterhouse, to which 3.2% sodium citrate was added as anticoagulant at the ratio by 9: 1(v/v) immediately after collection and used for fibrinogen and thrombin extraction.

Fibrinogen and thrombin were extracted by the methods of Futami et al.(1984) and Divakaran(1982), respectively. And the activity of thrombin was determined according to the method described by Abe(1961). The commercial products were used to confirm the fibrinogen and thrombin components in the extracts from the porcine blood by SDS-PAGE.The solution of thrombin and fibrinogen mixture(1:20,v/v) was dropped on the wound and added with 0.25Mcalcium chloride to induce clotting and form a clot and record it clotting time. New Zealand white rabbits were used as a testing animal to study haemostatic function of clotting agent. The blood vessels of both ears of the testing animals were cut surgically for bleeding and then dropped with clotting agent on the wound surface, separately and their coagulating time was recorded which was used to indicate the haemostatic function. The yield% and microbial counts were also determined.

Results and Discussion

The yield% of fibrinogen and thrombin were listed in Table 1 and the total bacterial counts and pH value of fibrinogen and thrombin were as Table 2. The microbial counts of 3.41 and 3.9cfu/g for fibrinogen and thrombin, respectively may be due to post contamination, however, the sterilization and effectiveness of the products will be studied in the future. The SDS-PAGE patterns of fibrinogen and thrombin were shown in Fig. 1 and 2, respectively. The activity of thrombin was shown in Table 3 and activity was the same as the result of Huang et al.(1992).

The haemostatic function of the solution was very effective when it was dropped on the wound surface of injured ear as compared to the control. The clotting times were from 2 to 15 seconds which depended upon the length of surgical cuts. However, the clotting time for the control(naturally clotting without dropping the clotting agent) was longer than the treatment. It took longer than 7 minutes. The experiment is still carried on to study effectiveness and to assure sterilization and abolishment antigenic properties of the product applied to surgical treatment of human kind.

Conclusions

The clotting agent prepared from thrombin and fibrinogen added calcium chloride from porcine blood can be used to arrest bleeding.

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Table 1 Yield% of Fibrinogen and Thrombin Extracted from Porcine Blood

	1st	2nd	3rd	Average
Fibrinogen	1.63	1.5	1.85	1.66
Thrombin	2.61	2.24	2.3	2.38

Table 2 Total Bacterial Counts and pH Value of Fibrinogen and Thrombin

	Total Bacterial Counts(cfu /g)	pH Value
Fibrinogen	3.41	6.75
Thrombin	3.9	6.97

Table 3 Activity of Thrombin

Dilution Time	5X	10X	15X	20X	25X**
Coagulation Time(5 min)	O	O	O	O	× 6 min

** If diluted 25X, the coagulation time became 6 min.
O Indicated completely coagulated within 5 min.
Thrombin activity : Unit of thrombin activity / ml = dilution time × 5.
Dilution time : Diluted with saline solution
5 : Dilution time in test tube.
Thrombin activity is expressed as the dilution time of 1 ml certain diluted thrombin required to clot 1 ml fibrinogen solution containing 7.5 mg in 5 min at 37 .
Therefore the thrombin activity is express as 20 × 5 = 100 unit.

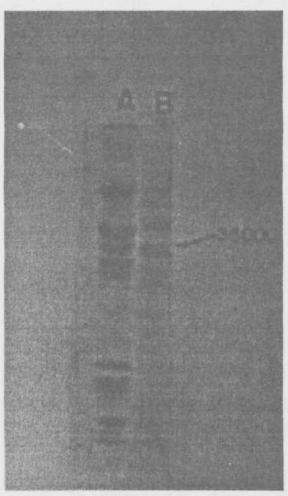

Fig1. SDS-polyacrylamide gel electrophoretogram of thrombin

A- Thrombin of porcine plasma.
B- Thrombin of bovine plasma-commercial

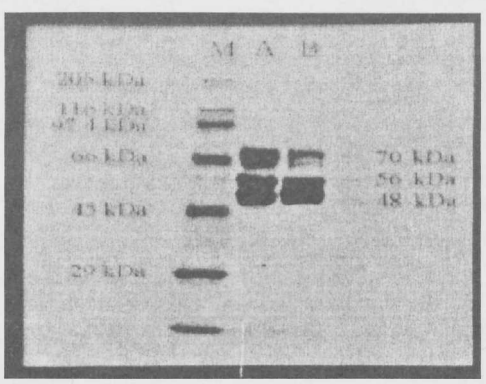

Fig2 SDS-PAGE electrophoretogram of pig plasma fibrinogen extracted by sodium citrate buffer.

Lane M-marker, Lane A -commercial fibrinogen-Sigma--
Lane B-fibrinogen extracted by sodium citrate buffer.