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EFFECT OF GAMMA IRRADIATION ON THE ENZYMATIC ACTIVITY AND THE HAEMOSTATIC FUNCTION OF FIBRINOGEN, THROMBIN AND FACTOR XIII

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Introduction

Plasma contains fibrinogen, thrombin and factor XIII which are blood coagulant factors. It has been found there are many valuable biomedical materials. In 2003, we extracted fibrinogen and thrombin from porcine blood as a haemostat solution and investigate its effectiveness of clotting. We found it had very effective haemostatic action (Chen *et al.*, 2003). Next year, we tried to use fibrinogen and thrombin to prepare haemostatic powder and film. The result revealed that the freeze-dried film was more porous and could arrest the bleeding effectively (Chen *et al*., 2004). This year, we study the additory effect of factor XIII on the haemostatic function of fibrinogen and thrombin plus calcium chloride. In order to obtain aseptic these coagulants, so the gamma irradiation is employed to sterilize the solution of fibrinogen, thrombin and factor XIII.

Objectives

This study is to investigate the additory effect of factor XIII on the haemostatic function of fibrinogen and thrombin plus calcium chloride, and the effect of gamma irradiation on the enzymatic activity and haemostatic action of the clotting agents.

Methodology

Fibrinogen, thrombin and factor XIII (transglutaminase) were extracted from porcine blood by the methods of Futami et al. (1984), Divakaran (1982) and Tseng (1999), respectively. These coagulating factors were freeze-dried to make powder which was irradiated by the dose of 3 KGy gamma ray (China Biotech Co.). The powder (without irradiation as the control) and the irradiated powder were diluted into solution by the ratio of 1:25 (powder : dist. water) for enzymatic activity analysis. The activities of thrombin and factor XIII of the control and the irradiated samples were determined by the methods of Abe (1961) and Folk (1970), separately. The solutions of thrombin and fibrinogen mixture (1:20) or plus factor XIII were dropped on the wound surface of ears of New Zealand white rabbit, then added with 0.25M calcium chloride to form a clot and

recorded its clotting time to indicate the haemostatic function. The effectiveness of irradiation on microbial counts was also determined.

Results & Discussion

The total microbial counts were found 2.16, 1.56 and 0.47 log cfu/g for the control samples of fibrinogen, thrombin and factor XIII, and zero for the irradiated samples, respectively (table 1). The result revealed that the dose of 3 KGy of gamma ray could kill the microorganisms presented in/on the powder of coagulating factors. Table 2 showed the effects of irradiation on enzymatic activities of thrombin and factor XIII. The results were found there was no difference in enzymatic activities between the control and the irradiated samples. The haemostatic effectiveness of fibrinogen(F)+ thrombin(T)+Ca was compared to fibrinogen+thrombin+Factor XIII(Ta).The result revealed the clotting time of the control(w/o coagulants) was 1.6X of F+T+Ca and 2.6X of F+T+Ta+Ca.(table 3). Thus, it was found factor XIII had an additory effect on the haemostatic function of fibrinogen and thrombin and Ca. The picture 1 was a clotting test of rabbit.

Conclusions

Cold sterilization could be used to kill the organisms presented in/on the coagulating factors, and this process did not affect the enzymatic activities of thrombin and factor XIII. It was also found the factor XIII had an additory effect on the haemostatic function of fibrinogen+thrombin added with Ca.

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Tables and Figures

Table 1 Effect of gamma-irradiation on the total microbial counts of fibrinogen, thrombin and factorXIII extracted from porcine blood

group	Fibrinogen	Thrombin	FactorXIII
Control (log CFU/g)	2.16	1.56	0.47
irradiation (log CFU/g)	0	0	0

Table 2 Effect of gamma-irradiation on enzymatic activities of thrombin and factor XIII

group	Thrombin	FactorXIII
Control	110 unit	0.016
irradiation	110 unit	0.017

Table 3 Clotting test of haemostat applied on New Zealand white rabbit

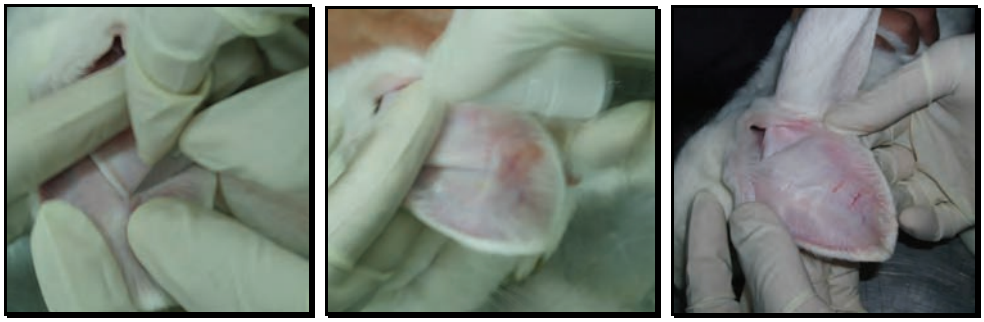
Group	Control	F+T+Ca	F+T+TG+Ca
Clotting time(second)	156	92	60

1. Control=without applying clotting agents.
2. F=fibrinogen, T=thrombin, TG=factorXIII and Ca=calcium.

Picture 1. Clotting Test on Animal

Control:

F+T+Ca:



F+T+TG+Ca:

1.Control=without applying clotting agents.
2.F+T+Ca=Fibrinogen+Thrombin+Calcium
3.F+T+TG+Ca=Fibrinogen+Thrombin+Factor XIII+Ca