

Role of ricin in platelet aggregation

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ABSTRACT

Ricin is a Ribosome Inactivating Protein from the seeds of *Ricinus communis*. The long time interest in this protein is due to the wide medical and therapeutical applications. It acts as a cytotoxic protein which has implications in cancer therapy. In this study we have established the role of ricin as an inhibitor of platelet aggregation. Platelet aggregation studies were done by turbidometric method and platelet inducing factors like ADP and collagen were used. Increasing concentrations of ricin were used on ADP induced and collagen induced platelets separately, to understand the role of ricin in platelet aggregation. Maximum inhibition was seen at 25µg/ml concentration of ricin.

Key words: Ricin, ADP, collagen, ribosome-inactivating protein.

INTRODUCTION

Ricin toxin is a heterodimeric 62Kda glycoprotein produced in the seeds of *Ricinus communis*. (Barbieri *et al.*, 1993). This protein is capable of inactivating ribosomes and thereby called Ribosome-Inactivating Protein (RIP). (Hartley *et al.*, 1996; Olsnes & Pihl 1973; Olsnes *et al.*, 1994). This RIP brings about halt in protein synthesis leading to cell death. Hence it has therapeutical application as antitumour protein (Nicolson GL, Blaustein J, 1972).

Platelet aggregation can be initiated invitro by various agonists like ADP, collagen, epinephrine etc. It is a multistep process. The analysis can be made using a turbidometer, by recording the tracing of changes in light transmission. During the initial phase of platelet aggregation following the addition of agonist like ADP or collagen, there is change in the shape of platelets which can be detected by a decrease in light transmission. (Kini & Evans H.J, 1990)

In the second stage, there is increase in light transmission as the platelets aggregate and

clear the way for the light to be transmitted through a platelet suspension. ADP and collagen induces platelet aggregation.

MATERIAL AND METHODS

ADP, collagen were purchased from Sigma chemicals Co. St Louis USA. Human blood samples were from healthy volunteers. All the chemicals used were of analytical grade. Ricin was purified in our laboratory.

Isolation of platelets

Human blood was collected from the vein of healthy non-smoker volunteers, who were not on any medication for at least the previous 2 weeks. Nine volumes of blood was collected into one volume of acid citrate dextrose (93mM sodium citrate, 7mM citric acid and 140mM glucose, p^H 6.5). and immediately centrifuged for 10min at 90Xg at room temp (25°C). The supernatant obtained was collected as Platelet Rich Plasma (PRP). Homologous platelet poor plasma (PPP) was prepared by recentrifugation of the remaining blood for 15min at 500Xg at room temp. The platelet concentration of PRP was adjusted with PPP to

2,25,000±25,000 platelets/µl. The PRP was kept at 37°C and was used within 2hrs after preparation. All the above preparations were carried out using plastic or siliconized glassware.

For platelet aggregation studies, the turbidometrical method (Born 1962) was followed using a chronology dual channel aggregometer. The recorder, recorded the light transmission as a function of time. When platelet aggregating agent was added to Platelet Rich Plasma (PRP), the platelets clumps and PRP becomes progressively clear showing increase in light transmission through it. Aliquots of PRP (0.45 ml) were placed in a siliconized glass cuvette in the aggregometer, positioned on a thermostat block at 37°C and continuously stirred by means of a small metal stirbar in the cuvette and a rotating magnet beneath. The turbidity of the PRP was constantly measured by recording transmission of a light beam directed through it and is recorded as change in voltage on the recorder. As platelets aggregate in response to an added agonist, light transmission increases progressively, producing an "Aggregation trace" on the recorder. This aggregation trace is the plot of light transmission between PRP and PPP baseline which represent 0% and 100% aggregation respectively. This curve is plotted as a function of time on a linear scale. For concentration dependent

platelet aggregation studies, different concentration (0-24µg/ml) of Ricin was incubated separately with PRP with constant stirring for 2min. aliquots of PRP were pre-incubated with Ricin. Platelet aggregation of treated PRP was then initiated by the addition of ADP(76µM), and collagen (2µg/ml) separately. Percent inhibition of aggregation was calculated assuming the value of control(without any aggregation inhibitor) to be 100%.

RESULTS AND DISCUSSION

Effect of Ricin on platelet aggregation was determined using PRP sample. Ricin inhibits platelet aggregation induced by each of the agonists ADP(Fig. 1) and collagen (Fig. 2). The inhibition was dose dependent with both the agonist induced platelet aggregation. Maximum inhibition was seen at 25µg/ml concentration.

Platelet aggregation is induced by large no of physiological agents such as ADP, serotonin, epinephrine, Collagen etc. The platelets interact through a specific receptor mechanism which mediate shape change aggregation and release reactions.(Kini & Evans H.J, 1990). In the present investigation the effect of Ricin on platelet aggregation proved ricin to be an inhibitor of platelet aggregation.

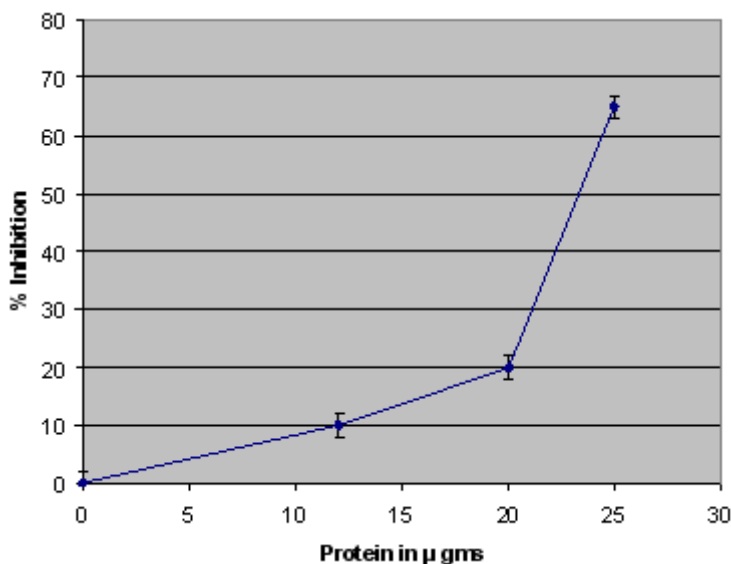


Fig. 1: Effect of Ricin on ADP induced platelet aggregation. Each value represents mean ± S.E.M of 3 experiments

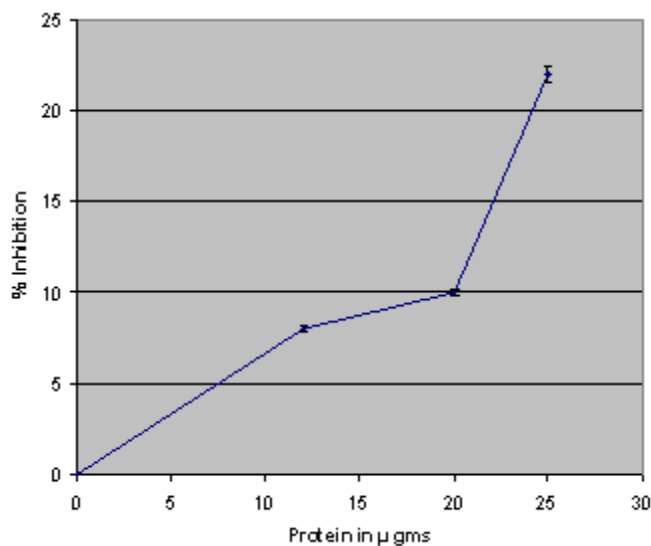


Fig. 2: Effect of Ricin on Collagen induced platelet aggregation.
Each value represents mean \pm S.E.M of 3 experiments

Some naturally occurring toxins have various pharmacological importance. Some of the toxins from snake venom for ex *Agkistrodon acutus* PLA₂ and *Agkistrodon halys* PLA₂ inhibit platelet aggregation. (Chen and Chen, 1989, Ouyang & Huang (1986), Huan G and Leecy 1984).

This pharmacological property of Ricin as an inhibitor of platelet aggregation can be further exploited in the development of prototypes of a new pharmaceutical agents. Understanding the mechanisms will help in the development of new research tools which are useful in characterising platelets aggregation at molecular level.

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