The Effect of Vitamin E on the Adenosine Diphosphate Induced Platelet Aggregation

ICHIRO KUROKAWA,* TOSHIYUKI KIMURA,* TATSUO NAGAI* AND MITSUO KAMIMURA**1

*Central Clinical Laboratory, Sapporo Medical College Hospital, Sapporo (Post No. 060) and **Department of Dermatology, Sapporo Medical College Hospital, Sapporo (Post No. 060)

(Received July 15, 1971)

The influence of vitamin E on the adenosine diphosphate (ADP) induced platelet aggregation was studied, and the following results were obtained. In these experiments, the solutions of \( \alpha \)-tocopheryl acetate (2.5 mg/ml) and \( \alpha \)-tocopheryl acetophosphate (2.5 mg/ml) were used.

1) When 0.5 mg of \( \alpha \)-tocopheryl acetate was added per 1 ml of platelet rich plasma (PRP) 2 hours prior to experiment, it inhibited ADP (which was added 1 \( \mu \)g per 1 ml of PRP) induced platelet aggregation.

2) Addition of 0.15 or 0.3 mg of \( \alpha \)-tocopheryl acetophosphate per 1 ml of PRP just prior to experiment evidently inhibited ADP induced platelet aggregation.

3) Addition of 0.1 or 0.2 mg of \( \alpha \)-tocopheryl acetate per 1 ml of PRP rather promoted the platelet aggregation induced by ADP. The same effect was also observed when the solvents of \( \alpha \)-tocopheryl acetate solution (equivalent volumes to 0.1, 0.2 and 0.5 mg \( \alpha \)-tocopheryl acetate) were added.

Some authors reported that \( \alpha \)-tocopheryl phosphate (referred as Toc-p) influences on several blood coagulation activities and that vitamin E has effects for thrombotic diseases (1-4). These facts suggest that vitamin E may affect not only on blood coagulation but also on the platelet function.

Recently, we found that \( \alpha \)-tocopheryl acetate (referred as Toc-ac) inhibited time dependant decrease of platelet number in the preserved blood with EDTA-K2 as anticoagulant (5).

In the present paper, the effect of Toc-ac and \( \alpha \)-tocopheryl acetophosphate (Toc-acp) on the ADP induced platelet aggregation is reported.

MATERIALS AND METHODS

1) PRP (platelet rich plasma); it was prepared from blood of normal person, men and women aged about 20, according to the following method. Forty five milliliters of blood was collected from each person by the siliconized glass syringes in which 5 ml of 0.106 molar trisodium citrate as anticoagulant was already contained. The collected materials were transferred into siliconized glass tubes followed by centrifuging 1,000 rpm for 5 minutes. Supernatant PRP were transferred to another siliconized glass tubes and kept at 4°C until experiment is started.

1 黒川一郎，木村寿之，永井龍夫，神村瑞夫
2) PPP (platelet poor plasma); it was prepared by further centrifugation of the residual blood at 3,000 rpm for 10 minutes.

3) Toc-ac solution; 2.5 mg/ml of Toc-ac in physiological saline solution was prepared by diluting the commercial preparation of Toc-ac solution (50 mg/ml).

4) Toc-acp solution; 2.5 mg/ml of Toc-acp solution was prepared from powdered form preparations.

5) ADP solution; 20 μg/ml in physiological saline solution was prepared.

6) Method of platelet aggregation; to measure the effect of vitamin E on the ADP induced platelet aggregation, the spectrophotometric method mainly according to Born (6) and Kloeze (7) was applied. Spectrophotometer used in the present experiment was Shimadzu-Bauschlohm spectrophotometer 20. The grade and duration of platelet aggregation was measured by the changes of optical density (OD) at 610 mμ. Initial OD of PRP was adjusted about 0.6 by diluting with its own PPP. PRP in a siliconized round cuvette was always agitated by a magnetic stirrer (5 mm in length and 1 mm in width of a small iron fragment wrapped with parafilm) and kept about 30° in a thermostat water bath during the estimation.

Three twentieth milliliter of the ADP solution was added to 3 ml of PRP (the concentration of ADP became 1 μg per 1 ml of PRP). The changes of OD of the mixture was measured every 30 or 60 seconds for 20 minutes since the addition of ADP solution.

In order to evaluate the effects of Toc-ac and Toc-acp, they were added to PRP (0.1, 0.2 or 0.5 mg of Toc-ac per 1 ml of PRP was added 2 hours prior to the addition of ADP, whereas 0.15 or 0.3 mg of Toc-acp per 1 ml of PRP was added just prior to the addition) and then the procedure was carried out.

The following two groups were used for control experiments:

a) Control “a”—Neither Toc-ac nor Toc-acp was added;

b) Control “b”—Solvent of Toc-ac solution was added. Their added volumes were corresponded to those of Toc-ac solution.

Vitamin E compounds and ADP used in the present experiment were the products of Eisai Co. (Japan) and Sigma Co. (USA) respectively.

RESULTS

1) The Effect of α-Tocopheryl Acetate

The data of addition of Toc-ac varying the amounts were given in Fig. 1.

1) Addition of 0.1 mg of Toc-ac per 1 ml of PRP

OD of the control “a”, control “b” and Toc-ac added groups were decreased 34% for 3 minutes (0.55±0.34), 54% for 12 minutes (0.56±0.24) and 48% (0.55±0.28) after addition of ADP, respectively. The decreased ratios of OD in the latter two groups were significantly more than that of control “a”.

2) Addition of 0.2 mg of Toc-ac per 1 ml of PRP

The decreased ratios of OD in the control “b” and Toc-ac added groups were 46% for 6 minutes (0.53±0.29) and 46% for 4 minutes
FIG. 2 The effect of $\alpha$-tocopheryl acetophosphate on the ADP induced platelet aggregation

- $\bigcirc$, addition of ADP alone 
- $\Delta$, 0.14 mg
- $\bullet$, 0.3 mg

(0.54–0.29) after addition of ADP, respectively.

These results showed that the addition of 0.2 mg of Toc-ac seemed to promote the ADP induced platelet aggregation less than that of 0.1 mg addition.

3) Addition of 0.5 mg of Toc-ac per 1 ml of PRP

OD of the control "b" and 0.5 mg of Toc-ac added group were decreased 47% (0.54–0.29) and 17% (0.49–0.41), respectively. These results showed that the Toc-ac added group partially inhibited the ADP induced platelet aggregation as compared with control "a" and "b" groups.

(2) The Effect of $\alpha$-Tocopheryl Acetophosphate (Fig. 2)

The decreased ratios of OD in the control "a", 0.15 mg and 0.3 mg of Toc-acp added groups were 25% for 4 minutes (0.61–0.46), 8% (0.58–0.53) and 8% (0.61–0.56), respectively. The addition of Toc-acp in both cases clearly inhibited the platelet aggregation.

DISCUSSION

From these results, it was shown that the addition of certain amounts of Toc-ac and Toc-acp inhibited the ADP induced platelet aggregation.

It was mentioned that ADP induced platelet aggregation might be caused by direct action of ADP on platelet membrane or the decrease of thrombosthenin (Ca-Mg-ATPase) activity derived from the decrease of superficial negative charges.

The platelet aggregation is reversible in the addition of rather small quantities of ADP, while it becomes irreversible like as that by the addition of thrombin, and ADP are discharged from inner side of platelets when ADP addition is increased.

The authors assumed in the previous report that the preserving effect of Toc-ac on the leucocytes in preserved bloods might be due to stabilization of lysosomal membrane in the leucocytes to a certain extent.

On the other hand, it was reported that vitamin A, which seems to show opposite effects of vitamin E in several biological activities, discharges acid-phosphatase and $\beta$-glucuronidase from platelets with the coexistence of dimethylsulphoxide.

Authors’ results showed that the addition of 0.1 or 0.2 mg of Toc-ac (fat soluble E) and equivalent volumes of the solvent for Toc-ac solution (which is surface active agent, classified as lysosome labilizer) promoted the ADP induced platelet aggregation. However, the addition of 0.5 mg of Toc-ac per 1 ml of PRP inhibited the aggregation despite of the promoting effect of the solvent. From these results, it was assumed that both the solvent and ADP might promote platelet aggregation, whereas Toc-ac might protect these effects. The mechanisms of this phenomenon have not been clarified. However, it was recently reported that Toc-ac increases the platelet number of idiopathic thrombocytopenic patients, so it may be noteworthy to promote further investigations on the effects of Toc-ac for the platelet aggregation.

On the effects of Toc-acp, authors reported that it inhibited blood coagulation by antithromboplastic and antithrombic action. On the other hand, platelets may be caused to agglutinate by thrombin. So, it is deduced that the inhibitory action of Toc-acp on the ADP induced platelet aggregation may be attributed partly to its antithrombic action.

Jackson et al. previously reported that alphatocopheryl phosphate (referred as Toc-p) inhibited platelet aggregation caused by the serum from idiopathic cytopenic patient.
Such result may still more support authors supposition since these two compounds resemble with each other in the chemical structural formula and solubility in water.

Acknowledgement

The authors wish to express their thanks to Prof. Fukushima (Chief of this central clinical laboratory) for his constant encouragement and criticism.

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