Prostaglandins and Medicine 4: 79-85, 1980

INHIBITION OF HUMAN PLATELET CYCLOOXYGENASE BY ALPHA-TOCOPHEROL

Muslim Ali, C. Gary Gudbranson and John W.D. McDonald
Department of Medicine, University of Western Ontario
London, Ontario. Canada. N6A 5C1 (Reprint Requests to JWDMc)

ABSTRACT

Alpha-tocopherol, an inhibitor of platelet aggregation, was evaluated for its effects on the synthesis of thromboxane and prostaglandins. A dose-dependent reduction in thromboxane B_2 and prostaglandin D_2 synthesis was observed with approximately 60% inhibition at 5.0 IU of alpha-tocopherol. Alpha-tocopherol produced a time-dependent, irreversible inhibition.

INTRODUCTION

A number of nonsteroidal antiinflammatory drugs are known to be potent inhibitors of platelet cyclooxygenase activity (1-3). Most common of them are the aspirin-like drugs which inhibit the formation of prostaglandins (4,5). A number of antioxidants such as alpha-napthol,butylated hydroxy toluene, D-alpha-tocopherol (αT) and nitroblue-tetrazolium also inhibit prostaglandin formation (6,7). αT is the only naturally occurring biological antioxidant in the human body. It inhibits aggregation of human platelets induced by collagen, epinephrine, thrombin, ADP and arachidonic acid (8,9).

αT prevents the formation of peroxides in adipose tissue *in vivo* (10). Higashi *et al.* suggested that αT inhibited in platelets at the level of lipid peroxidation initiated by hydrogen peroxide (11). Its efficacy in reducing the rate of lipid peroxidation in stored platelet suspensions has been reported previously (12). The mechanism by which αT inhibits platelet aggregation was shown to be associated with its inhibitory effect on the release of calcium from platelet membrane *in vitro* (13). Platelet hyperaggregability noticed in two children who were αT deficient was corrected by αT therapy (14). The peroxidation product malondialdehyde was increased in plasma in association with the αT-deficient state but fell to normal levels once αT supplementation began. This suggests that αT acts at the step of phospholipase "A" activation or on cyclooxygenase activity.

In the present study we have investigated the role of αT as an inhibitor of

synthesis of thromboxane B_2 (TXB_2) and prostaglandin D_2 (PGD_2) synthesized from arachidonic acid by washed human platelets *in vitro*.

METHODS

Succinate ester of d-α-tocopherol (1210 IU/g, Sigma Chemical Company, St. Louis, Mo.) was dissolved in ethanol. The insoluble residue was removed by centrifugation. The residue was resuspended in ethanol and assayed for activity in parallel with the supernatant fraction. All inhibitory activity was confined to the supernatant fraction. All preparative operations were performed under nitrogen and the αT stock solution was stored under nitrogen at 4 °C.

Arachidonic acid-1-^{14}C (5,8,11,14-eicostetraenoic acid-^{14}C, specific activity 58 mci/mmol, Amersham-Searle Corporation) was mixed with unlabelled arachidonic acid (Sigma Chemical Company, St. Louis, Mo.) stored and used as described previously (2).

Washed human platelet suspensions were prepared following the method of Wolfe and Shulman (15). Blood was drawn from healthy donors who had not taken any nonsteroidal antiinflammatory drugs or αT for at least 10 days. A final suspension of washed platelets (1×10^9/ml) was prepared in 0.05M Tris-HCl buffer, pH 7.4, containing 0.015M Na_2EDTA.

Temperature equilibration of 0.25 ml of platelet suspension was carried out for 5 minutes at 37 °C. Following the addition of 0.01 ml of ethanol or αT solution, preincubation was continued for 5 minutes. Prostaglandin synthesis was initiated by the addition of arachidonic acid in a volume of 0.2 ml. The final ethanol concentration was less than 2% and had no effect on cyclooxygenase activity. All incubations were carried out in triplicate. After one minute incubation with arachidonic acid, a mixture of ethanol and saline (2:1) was added to stop the reaction. Chloroform extraction and the separation from precursor arachidonic acid of labelled TXB_2 and a small amount of PGD_2 on silicic acid columns were carried out as described previously (16). The fraction eluted in 6% methanol from the silicic acid columns was further fractioned on thin layer plates. The plates were developed in solvent system A, as described earlier (16). Radioactively-labelled reaction products on thin layer plates were detected using the Packard Radiochromatogram Scanner (Model 7201).

RESULTS

A time-dependent inhibition of cyclooxygenase activity was observed in the presence of αT. Maximum inhibition was observed after 5 minutes preincubation with αT (Figure 1). Subsequent experiments were carried out using 5 minutes preincubation.

Figure 2 shows a radiochromatogram scan of a thin layer chromatogram of reaction products eluted from silicic acid columns. The major radioactive peak cochromatographed with authentic TXB_2 marker. A minor radioactive peak of PGD_2 was also observed. The TXB_2 peak was markedly reduced in the presence of αT (5 IU) (Figure 2).

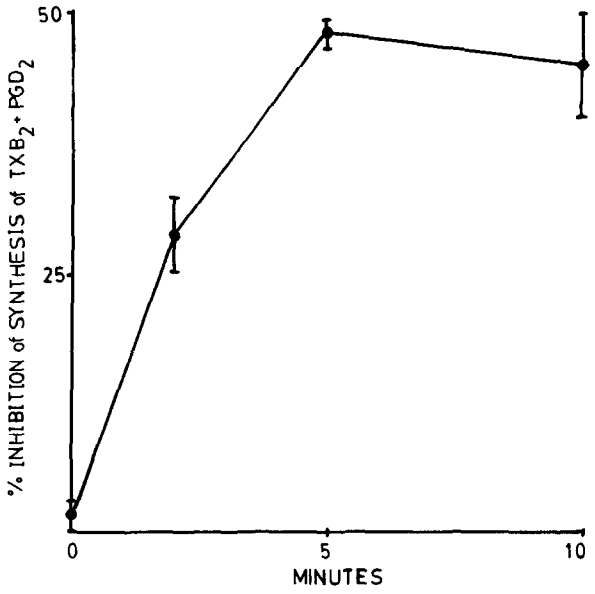

Figure 1: The effect of preincubation time with αT on synthesis of TXB_2 + PGD_2 by washed platelets. Synthesis of TXB_2 + PGD_2 was initiated by the addition of ^{14}C-arachidonic acid (10 μM) following preincubation with αT (5 IU) or with saline. Extracts for chromatography were prepared after one minute of incubation. Each point represents mean of 3 experiments ± S.E.M.

A dose-response curve of αT versus cyclooxygenase activity is shown in Figure 3. A dose-dependent inhibition of cyclooxygenase activity by αT was observed. Greater than 60% inhibition of TXB_2 and PGD_2 formation was observed at 5 IU of αT. Table 1 shows the effect of αT (1 IU and 5 IU) on the synthesis of TXB_2 and PGD_2 by platelets at various concentrations of arachidonic acid (5 to 50 μM). An irreversible type of inhibition by αT with respect to substrate (arachidonic acid) was observed. About 33 and 60% inhibition by 1 IU and 5 IU of αT was observed at all concentrations of arachidonic acid tested.

DISCUSSION

Our results with preincubation of αT with platelets (Figure 1) showing maximum inhibition reached in 5 minutes correlates well with work of Steiner and Anastasi (9) who have demonstrated the maximum uptake of αT by platelets in a 5 minute period.

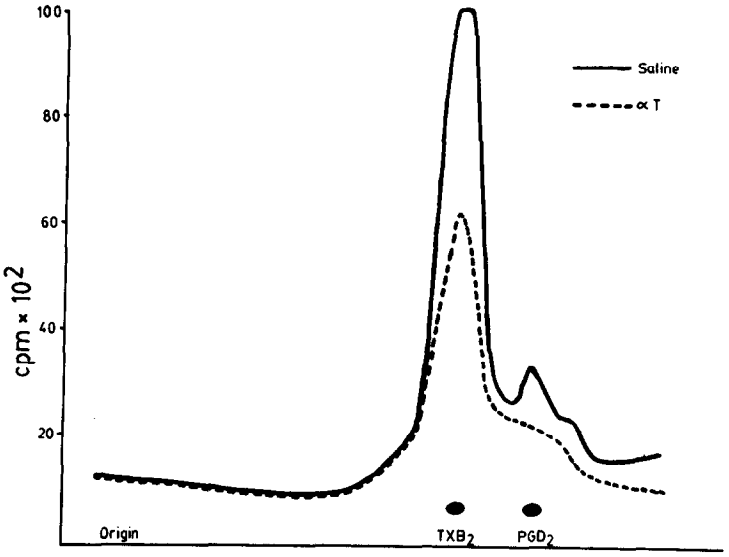

Figure 2: Radiochromatogram scan of thin layer chromatogram of radioactively-labelled products following incubation of washed human platelets with arachidonic acid-^{14}C.

Table 1: Effect of substrate concentration on inhibition of platelet TXB_2 + PGD_2 synthesis by D-α-tocopherol succinate. Synthesis of TXB_2 + PGD_2 from arachidonic acid-^{14}C during one minute incubation was determined after preincubation for 5 minutes with αT.

Arachidonic Acid Concentration (μM)	% Inhibition of TXB_2 + PGD_2 Synthesis Mean ± S.E.M.	
	αT (1 IU)	αT (5 IU)
5	36 ± 8 (3)*	56 ± 5 (4)
10	22 ± 7 (3)	52 ± 2 (4)
15	21 ± 6 (3)	48 ± 2 (4)
20	22 ± 6 (3)	48 ± 2 (4)
25	33 ± 17 (3)	53 ± 8 (4)
50	33 ± 19 (3)	61 ± 7 (4)

* Number of independent determinations

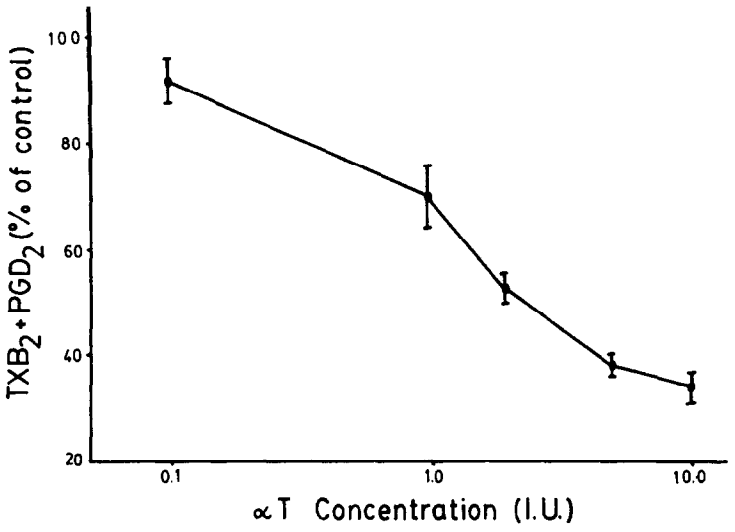

Figure 3: Effect of αT on synthesis of TXB_2 and PGD_2 by washed platelets. Synthesis of TXB_2 + PGD_2 from ^{14}C-arachidonic acid (10 μM) during one minute incubations was determined after preincubation for 5 minutes with D-α-tocopherol succinate. Results shown represent the mean of 4 independent experiments (± S.E.M.)

The results from Figure 1 and Table 1 in the present study show time-dependent irreversible inhibition by αT of synthesis of TXB_2 plus PGD_2 as has been described for aspirin and indomethacin (3,17). Synthesis of TXB_2 from cyclic endoperoxide (prostaglandin H_2) was not blocked in the presence of αT. This result indicates that αT exerts its inhibitory effect at the cyclooxygenase step rather than on thromboxane synthetase.

Our results differ from the findings of Rao *et al.* (18) who found no inhibitory effects of αT on cyclooxygenase activity of platelet microsomes. Rao *et al.* (18) did not preincubate the enzyme preparation with αT and used the acetate rather than the succinate form of the vitamins. The inhibition by αT of platelet aggregation may be due to its inhibitory effect on cyclooxygenase activity. The concentrations of αT which produced significant inhibition of platelet cyclooxygenase activity are in a range which may be encountered in the plasma of subjects receiving approximately 1000-2000 IU per day (9).

ACKNOWLEDGEMENT

This work was supported by the Medical Research Council of Canada and the Ontario Heart Foundation.

The technical assistance of Mrs. Carolyn Armstrong is greatly appreciated.

REFERENCES

1. Zucker MB and Peterson J. Effect of acetylsalicylic acid, other non-steroidal antiinflammatory agents and dipyridamole on human blood platelets. J Lab Clin Med 76:66, 1970.

2. Ali M and McDonald JWD. Effects of sulfinpyrazone on platelet prostaglandin synthesis and platelet release of serotonin. J Lab Clin Med 89:868, 1977.

3. Ali M and McDonald JWD. Reversible and irreversible inhibition of platelet cyclooxygenase and serotonin release by nonsteroidal antiinflammatory drugs. Thrombosis Research 13:1957, 1978.

4. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biology 231:232, 1971.

5. Smith JB and Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. Nature New Biology 231:235, 1971.

6. Panganamala RV, Miller JS, Gwebu ET, Sharma JM and Cornwell DG. Differential inhibitory effects of vitamin E and other antioxidants on prostaglandin synthetase, platelet aggregation and lipoxidase. Prostaglandins 14:261, 1977.

7. White JG, Rao GH and Gerrard JM. Effects of nitroblue tetrazolium and vitamin E on platelet ultrastructure aggregation and secretion. Amer J Path 88: 387, 1977.

8. Fong JSC. Alpha-tocopherol: its inhibition on human platelet aggregation. Experimentia 32:639, 1976.

9. Steiner M and Anastasi J. Vitamin E - an inhibitor of the platelet release reaction. J Clin Invest 57:732, 1976.

10. Tappel AL. Vitamin E as a biological lipid antioxidant. Vitamins and Hormones 20:493, 1962.

11. Higashi O and Kikuchi V. Effects of vitamin E on the aggregation and the lipid peroxidation of platelets exposed to hydrogen peroxide. Toloku J Exp Med 112:271, 1974.

12. Okuma M, Steiner M and Baldini M. Studies on lipid peroxides in platelets. 1. Method of assay and effect of storage. J Lab Clin Med 75:283, 1970.

13. Butler AM, Gerrard J, Peller J, Stoddard SF, Rao GHR and White JG. Vitamin inhibits the release of calcium from a platelet membrane fraction *in vitro*. Prostaglandins and Medicine 2:203, 1979.

14. Lake AM, Stuart MJ and Oski FA. Vitamin E deficiency and enhanced platelet function-reversal following vitamin E supplementation. J Pediatrics 90:722, 1977.

15. Wolfe SM and Shulman NR. Adenylcyclase activity in human platelets. Biochem Biophys Res Commun 35:265, 1969.

16. Ali M, Cerskus AL, Zamecnik J and McDonald JWD: Synthesis of prostaglandin D_2 and thromboxane B_2 by human platelets. Thrombosis Research 11:485, 1977.

17. Roth GJ, Stanford N and Majerus PW: Acetylation of prostaglandin synthase by aspirin. Proc Nat Acad Sci USA 72:3073, 1975.

18. Rao GHR, Gerrard JM, Eaton JW and White JG. Arachidonic acid peroxidation, prostaglandin synthesis and platelet function. Photochemistry and Photobiology 28:854, 1978.