

ENHANCED PLATELET AGGREGATION BY ORAL CONTRACEPTIVES : EFFECT OF PG SYNTHETASE INHIBITORS

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(Received on March 1, 1982)

Summary : Platelet aggregation time was significantly ($P < 0.01$) decreased in female rabbits treated with oral contraceptive (a preparation containing low dose of estrogen) as also by injection of diethylstilbestrol (10 mg/kg), while in animals that received indomethacin (10 mg/kg) or aspirin (30 mg/kg) (PG synthetase inhibitors) alongwith oral contraceptives or diethylstilbestrol there was no significant alteration in platelet aggregation time. The increased synthesis of prostaglandins or some of the intermediary product like TXA₂ might be responsible for this effect.

Key words : platelet-aggregation

estrogen

PG synthetase inhibitors

INTRODUCTION

The association of thromboembolic disorders with the use of oral contraceptives has been well established (1, 2, 16), however, the mechanism still remains unclear. Some investigators have described the changes in plasma levels of various clotting factors (4, 5, 6) while others have demonstrated increased platelet aggregation (10, 13). Since platelets play an important role in thrombosis and atherogenesis (19), it is important to study whether oral contraceptives cause any change in platelet reactivity, aggregation and adherence. Recent studies have demonstrated increased platelet aggregation in females using contraceptives pills. However, there are conflicting reports regarding the role of prostaglandins in platelet aggregation. Some investigators have reported the anti-aggregatory role of prostaglandins (3, 8, 11) while others, have demonstrated pro-aggregatory response to prostaglandins or some of the intermediary products of arachidonic acid (15, 17).

Recently it has been observed that prostaglandins and some of the other intermediate products of arachidonic acid like thromboxanes play an important role in enhancing the platelet aggregation (19). It has also been observed that the synthesis of thromboxanes and prostaglandins is inhibited by indomethacin and other nonsteroidal anti-inflammatory drugs (NSAID) (7, 9, 20). However, a much work has not been

done on the mechanism of alteration of prostaglandin synthesis in response to estrogens. In the present study attempt is made to observe the effect of estrogens on platelet aggregation and to study the role of non-steroidal anti-inflammatory drugs in its prevention.

MATERIAL AND METHODS

The study has been carried out on female rabbits weighing 1-1.5 kg. Animals were divided into 7 groups of 10 each. The first group served as control. Rabbits of the second group received lynestrenol 1 mg+ ethinylestradiol 0.05 mg/kg daily for 8 weeks and those of third group received injection diethylstilbestrol (10 mg/kg, im) weekly for 8 weeks. The animals of the fourth and the fifth group were given indomethacin (10 mg/kg) and aspirin (30 mg/kg) respectively orally, alongwith oral contraceptives. The animals of the sixth and the seventh group were treated with indomethacin and aspirin respectively alongwith injection diethylstilbestrol.

At the end of the study, blood was withdrawn directly from the heart in siliconized vials containing 3.8% solution of sodium citrate in the ratio of 9:1. The platelet rich plasma (PRP) was obtained by centrifuging the blood samples at 150-200G for 15 min ADP (20 µg) induced platelet aggregation was observed by the method of O'Briens (12). The method is based on the principle that when ADP is added to vigorously stirred PRP, the formation of platelet clumps produces an alteration in the optical density which can be measured by making serial readings with a photoelectric colorimeter.

Three ml of PRP was taken in a siliconized tube and kept in the colorimeter with operative wave length of 550 mµ. The optical density reading was set at 0.40 and kept constant for every test. 0.1 ml of 200 µg/ml ADP solution was added to constantly stirred PRP. The optical density reading was noted every 5 sec and the results expressed as platelet aggregation time in sec. The aggregation time was observed for 30 sec since ADP induces immediate aggregation but the aggregates tend to disperse after 20 sec. The platelet aggregation time of all the groups was compared to that of the controls using paired "t" test.

RESULTS

The animals receiving oral contraceptive or diethylstilbestrol alone, exhibited a marked decrease in platelet aggregation time as compared to the control ($P < 0.01$), whereas those which received indomethacin or aspirin alongwith oral contraceptive or diethylstilbestrol, did not exhibit ($P > 0.05$) alteration in aggregation time. The results are depicted in Fig. 1.

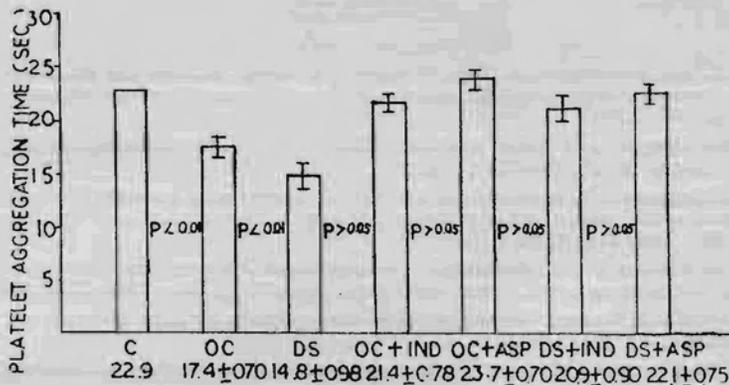


Fig. 1 : Effect of drugs on ADP-induced platelet aggregation.

- C = Control,
- OC = Lynestrenol 1 mg + ethinylestradiol 0.05 mg/kg.
- DS = Injection diethylstilbestrol, (10 mg/kg).
- IND = Indomethacin (10 mg/kg).
- ASP = Aspirin (30 mg/kg).

DISCUSSION

The results of the study demonstrate that estrogens cause enhancement of platelet aggregation. These findings support the reports of Bolton *et al.* (2), Bierenbaum *et al.* (1). It has further been observed that prostaglandins or some of the intermediary products of arachidonic acid like TXA₂ are possibly involved in causing enhancement of platelet aggregation due to estrogens, since the animals that were simultaneously treated with aspirin or indomethacin, (known inhibitors of PG synthetase) did not exhibit much alteration in platelet aggregation time as compared to the normals. These results are in agreement with the reports of Vane (20), Malmsten *et al.* (9) and Subbiah *et al.* (19). The recent report of Subbiah *et al.* (19) describes enhancement of platelet aggregation in female pigeons treated with diethylstilbestrol. It further indicates an increase in the level of TXB₂ which is a stable metabolite of TXA₂ - a proaggregatory factor.

Aspirin causes irreversible acetylation of the platelets which may last for as long as 11 days (14). In the present study aspirin and indomethacin were equally effective in the dose used although a greater efficacy of aspirin was expected.

It seems the NSAID act at the hydrophobic site of the oxygenase system i.e. cyclooxygenase. These drugs interfere with the prostaglandin biosynthesis by inhibiting the synthesis and not the breakdown of the cyclic endoperoxides (18).

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