Preliminary Communications

Counteraction of Platelet Activity at Sites of Laser-induced Endothelial Trauma

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Summary: The effects of dextran 70 (Mw 70,000; Macrodex), heparin, dipyridamole (Persantin), and prostaglandin E_1 on the behaviour of platelets were investigated at sites of endothelial trauma induced with a ruby biolaser in arterioles in the rabbit ear chamber. This technique has several advantages over previous methods of studying platelet activity.

Dextran 70 (2 g./kg. body weight) caused a profound reduction in platelet activity four to six hours after its administration. Heparin (12 mg./kg. body weight) had no effect. The effect of dipyridamole (2.5 mg./kg. body weight) was profound immediately after its administration, but was transient. In preliminary experiments single intravenous injections of prostaglandin E_1 (25 and 125 μ g./kg. body weight) had no detectable effect.

INTRODUCTION

There is much to suggest that platelet activity underlies the initiation and propagation of thrombi. Interest is therefore turning towards the experimental and clinical evaluation of drugs that diminish platelet adhesiveness and aggregation in patients liable to develop thrombosis or in whom it is established. Precise methods of measuring platelet function in vitro are available, but measurement in vivo has been difficult. Recently a reproducible in-vivo method was described (Arfors *et al.*, 1968). A laser is used to inflict precise endothelial trauma in arterioles in the rabbit ear chamber, and observations are made on the subsequent platelet activity. This method has been used in a preliminary investigation of the effects of dextran 70 (Mw 70,000; Macrodex), heparin, dipyridamole (Persantin), and prostaglandin E_1 on platelet behaviour at sites of endothelial trauma.

MATERIALS AND METHOD

Conscious Sandy-Lop rabbits with regenerative ear chambers were used. The microvasculature was observed at a magnifica-

tion of $\times 230$. Vessels with a luminal diameter of 20-30 μ showing rapid arteriolar flow were injured with a single-pulse ruby biolaser (model 153, T.R.G., N.Y.).

It was previously shown that after biolaser endothelial trauma with an energy input of 180-200 joules the cumulative number of platelet microemboli from the damaged site (about 6 μ in diameter) in 15 minutes is reproducible, and therefore suitable for measuring platelet activity (Arfors *et al.*, 1968).

Experiments were performed on six rabbits. In each at least two control tests were made: the number of emboli from sites of fresh injury was counted for 15 minutes. Then the animals were given dextran 70, heparin, dipyridamole, or prostaglandin E_1 intravenously. The effects of these substances were measured by inflicting at least two additional fresh injuries at various times.

RESULTS

The results of a typical experiment are shown in Fig. 1. Control tests were made and the number of emboli was plotted against time. Heparin 12 mg./kg. body weight was given intravenously, and four to six hours later fresh injuries were inflicted. After heparin the rate of embolization was unchanged ($P=0.7$). Four days later further control tests showed in 15 minutes a similar number of emboli to that in the previous control tests. Dextran 70 (2 g./kg. body weight) was given intravenously, and the animal was tested immediately and four to six hours after infusion. Dextran had no immediate effect ($P<0.4$), but four to six hours later platelet activity was profoundly inhibited ($P\leq 0.001$).

Fig. 2 shows the mean results of six experiments in which rabbits given dextran 70 (2 g./kg. body weight) were tested with fresh injuries before and immediately after infusion and four to six hours and 24 hours later. Immediately after infusion platelet activity was relatively unchanged (93% of control; $P=0.4$), but a highly significant reduction was seen in every animal four to six hours after dextran (21% of control; $P\leq 0.001$). Platelet activity had returned almost to preinfusion values 24 hours after dextran ($P=0.15$).

In three animals given 12 mg. of heparin per kg. body weight and tested four to six hours later inhibition of platelet activity was not found.

Three animals were given dipyridamole (2.5 mg./kg. body weight) intravenously and tested before, immediately after, and

FIG. 1

FIG. 2

FIG. 3

FIG. 1.—Results of a typical experiment in one rabbit. The mean numbers of emboli formed in the control state and after heparin and dextran 70 are shown. The number of fresh injuries inflicted is given in parentheses. The shaded area outlines the 95% confidence limits of the control observations. FIG. 2.—Effect of dextran 70 on platelet activity at sites of fresh endothelial injuries (mean results ± 1 S.E. in six rabbits). FIG. 3.—Effect of dipyridamole on platelet activity at sites of fresh endothelial injuries (mean results in three rabbits).

20 to 30 minutes and two to three hours after its administration. Immediately after dipyridamole a pronounced reduction (Fig. 3) in the cumulative number of emboli was found (21.5% of control; $P \leq 0.001$). However, the effect was transient, and injuries inflicted 20 to 30 minutes after dipyridamole showed platelet activity comparable to that seen in the control tests (91% of control; $0.3 > P > 0.2$).

Neither of the two animals given prostaglandin E_1 (25 and 125 $\mu\text{g./kg.}$ body weight) intravenously and tested before, immediately after, and 45, 75, and 120 minutes after its administration showed an inhibitory effect on platelet activity.

DISCUSSION

With the technique used in the present investigation it is possible to study the effects of different materials on different days in the same animal. Each animal acts as its own control, thus overcoming the problem of the individual nature of platelet reactivity. The technique has additional advantages over the methods hitherto available. The animals are not anaesthetized, endothelial injury is precise and reproducible, and the observations are made at high magnification. Furthermore, platelet activity at sites of fresh laser injury at different times is remarkably constant (Arfors *et al.*, 1968).

Dextran 70 was shown to have a pronounced inhibitory effect on platelet activity at sites of endothelial trauma. Indeed, the changes were highly significant even in a single animal. Since four to six hours elapse before the effect of dextran becomes maximal it cannot be ascribed to dilution of platelets after volume expansion or to hyperkinetic blood flow. It is of interest that a similar delay is found in man after dextran infusion before the maximum effect on platelet behaviour as tested in vitro (Bennett *et al.*, 1966; Bygdeman *et al.*, 1966). This suggests the possibility that a time-dependent interaction between dextran and some plasma factor underlies the observed inhibition of platelet activity.

In contrast to dextran, other materials of current interest were found to be disappointing in this preliminary study. Heparin had no significant effect. The effect of dipyridamole was transient; though pronounced suppression of platelet activity was seen immediately after its administration, the activity had returned to control values after 20 to 30 minutes. The transient

effect of dipyridamole on platelet behaviour at sites of injuries inflicted after its administration has already been noted (Emmons *et al.*, 1965). However, there is evidence that platelet activity at sites of injuries inflicted before the administration of dipyridamole is profoundly suppressed (Emmons *et al.*, 1965). The reason for this difference is not clear.

The lack of effect after a single intravenous dose of prostaglandin E_1 given before injury may be accounted for by the rapid inactivation of prostaglandins in the lungs (Bergström and Samuelsson, 1965). Nevertheless, Emmons *et al.* (1967) claim that platelet activity is suppressed in vivo after similar single doses. Further work using single doses and continuous intravenous and intra-arterial infusion of prostaglandin E_1 is clearly necessary.

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REFERENCES

- Arfors, K.-E., Dhall, D. P., Engeset, J., Hint, H. C., Matheson, N. A., and Tangen, O. (1968). *Nature (Lond.)*, **218**, 887.
Bennett, P. N., Dhall, D. P., McKenzie, F. N., and Matheson, N. A. (1966). *Lancet*, **2**, 1001.
Bergström, S., and Samuelsson, B. (1965). *Ann. Rev. Biochem.*, **34**, 101.
Bygdeman, S., Eliasson, R., and Gullbring, B. (1966). *Thrombos. Diathes. haemorrh. (Stuttg.)*, **15**, 451.
Emmons, P. R., Harrison, M. J. G., Honour, A. J., and Mitchell, J. R. A. (1965). *Nature (Lond.)*, **208**, 255.
Emmons, P. R., Hampton, J. R., Harrison, M. J. G., Honour, A. J., and Mitchell, J. R. A. (1967). *Brit. med. J.*, **2**, 468.

Medical Memoranda

Uncommon Complication of Anaphylactoid Purpura

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Anaphylactoid purpura, the Henoch-Schönlein syndrome, is a common condition in childhood and one in which abdominal pain frequently occurs. This may on occasion be severe enough to mimic an acute surgical abdomen, and an unnecessary laparotomy may be performed. Recently two children suffering from anaphylactoid purpura were admitted to the Mansfield General Hospital, and in the course of this illness both boys developed testicular pain and scrotal swelling clinically simulating acute testicular torsion. In one patient exploration excluded torsion, and in both patients it was assumed that their symptoms were associated with the underlying condition and were not purely unrelated incidents.

CASE 1

A boy aged 5½ years was admitted to the children's ward on 10 February 1968 with symptoms typical of the Henoch-Schönlein syndrome. He had been well until two weeks before admission, when he developed a cough and signs of an upper respiratory tract infection. During the following week he complained of pains in his legs, and on the evening before coming into hospital it was noted that his ankles were swollen and he was unable to walk. His previous health had been good and he had been in hospital only once before, when he had a circumcision in 1967.

On examination there was a characteristic rash on his elbows, buttocks, and legs. Both feet were puffy and his ankles painful, the right more than the left. His temperature orally was 99° F. (37.2° C.) and the E.S.R. was reported as 20 mm. in the first hour. A throat swab was reported subsequently as showing no evidence of haemolytic staphylococcus. Urine examination showed no abnormality. The antistreptolysin O titre was 500 Todd units.

The child was prescribed penicillin and his progress over the next few days was satisfactory. The swelling of his feet gradually sub-