*Br. J. Pharmac.* (1980), **69**, 227–231

# THE EFFECTS OF VINCRISTINE ON PLATELET AGGREGATION STUDIED BY A FILTER LOOP TECHNIQUE IN THE RAT

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1 A method for measuring aggregation of platelets by adenosine diphosphate (ADP) is described using a filter inserted into the flowing aortic blood in the rat.

2 Repeated infusions of ADP resulted in a fall in the calculated aggregation index without significant changes in the platelet count.

3 Vincristine (0.05 mg/kg) intravenously caused significant inhibition of ADP-induced platelet aggregation.

4 Infusion of ADP caused some peripheral vasodilatation though it is unlikely that this contributed to the effects seen to any great extent.

## Introduction

The most commonly used method for assessing platelet function is the stimulation of platelet aggregation *in vitro* in an aggregometer (Born, 1962) although there is some doubt as to whether *in vitro* platelet aggregation reflects their behaviour *in vivo* (Packham & Mustard, 1977). Swank, Roth & Jansen in 1964 used a filter screen technique to assess platelet aggregation and this has been used by others (Fleischman, Bierenbaum & Stier, 1976). In 1970, Hornstra extended the filter method by inserting it into flowing aortic blood in the rat.

Vincristine, a vinca alkaloid, is used in the chemotherapy of malignant disease and is thought to prevent mitosis by disrupting the microtubular system. It has been shown that vincristine also disrupts the peripheral microtubular system in platelets studied *in vitro*, producing depressed aggregation but that clot retraction is unaffected by the drug (White, 1968; 1969).

We have simplified and modified Hornstra's technique to study the effects of vincristine on adenosine diphosphate (ADP)-induced aggregation in blood passing through a filter inserted into the aorta of the rat. Our preliminary results have been published (Martin, Suggett & Thwaites, 1978).

## Methods

Experiments were performed on adult male Wistar rats which were anaesthetized with ethyl carbamate (Urethane, BDH) 1.3 to 1.5 g/kg body weight intraperitoneally. A total of 8 control and 14 experimental animals were used in the vincristine experiment and the mean body weight of the 22 animals was 363 ± 11 g (s.e. mean). There was no significant difference between the weights of the animals in the control and experimental groups.

A polyethylene tracheal cannula (2 mm i.d.) was inserted and the animal heparinised (1000 units/kg) via a cannula in the femoral vein. The aorta was exposed through a midline incision and the abdominal contents displaced to one side.

The aorta was catheterized below the renal arteries both proximally and distally with 2.0 and 1.2 mm internal diameter stainless steel cannulae respectively, connected by polyethylene tubing to the chamber containing the filter (Figure 1). The chamber was constructed from perspex in two halves held together by a twist-lock mechanism with the filter (Veco Limited, Eerbeck, Holland) 15 mm in diameter supported betweeen a pair of rubber 'O' rings. The twist-lock closure was constructed from the locking ring of a standard locking DIN audio plug which was pushed tightly over one half and locked into milled recesses in the other.

The filter had pores 20 μm in diameter allowing the passage of all blood constituents. The apparatus was primed with saline and its total volume including cannulae was 0.9 ml.

ADP was infused proximal to the filter at rates of 0.17 to 17 μg/min by means of a Harvard Infusion Pump with a 2 ml syringe containing freshly prepared 50 μM ADP (Sigma Chemical Co). Infusions were continued for 30 s. Electromanometers were used to record proximal and distal pressures and the results recorded on an ultraviolet paper recorder (S.E. Laboratories Ltd). Rectal temperature was main-

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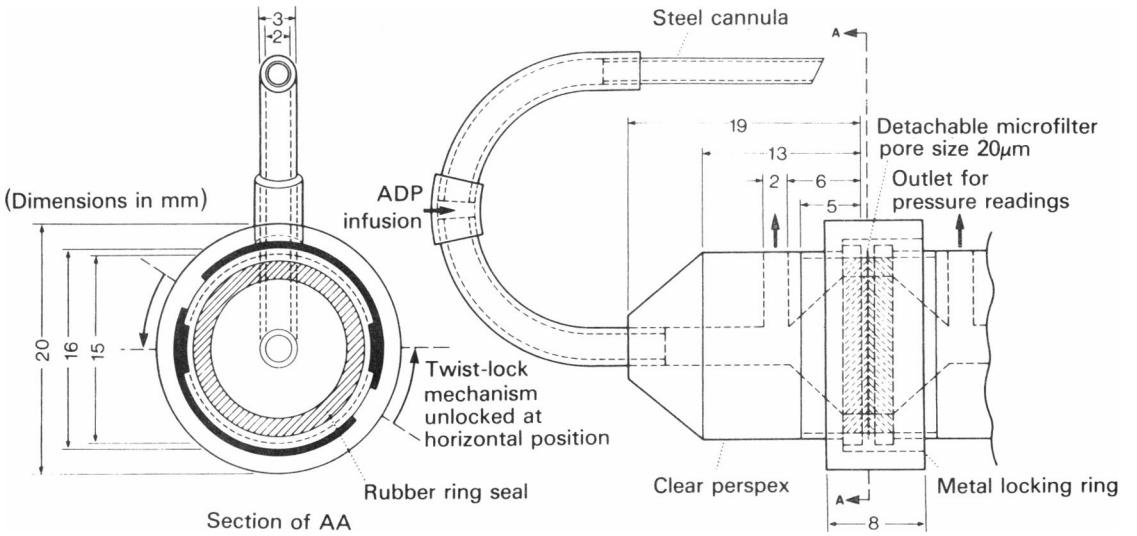


**Figure 1** Diagram of the chamber, connecting tubing and cannula that is inserted into the rat's aorta. The two units of chamber and tubing either side the filter are similar, except that adenosine diphosphate (ADP) is only infused into the proximal loop. The outlets for pressure readings are connected to electromanometers.

tained by gentle external heating if necessary and intravenous saline was not administered to restore blood pressure if at all possible.

A dose-response curve to ADP was recorded in each animal before either vincristine or saline was given intravenously. Test animals were given vincristine sulphate (Oncovin, Eli Lilly and Co.), 0.05 mg/kg whereas control animals were given a similar volume of saline. Five min were allowed to elapse after injection of vincristine or saline before a second dose-response curve to ADP was recorded in both test and control animals.

Platelet counts were performed in 4 control and 6 test rats before and after saline or vincristine respectively by a manual counting method.

To assess the effects of ADP on the hind limb circulation of the rat the distal aorta was perfused with blood at constant flow in 6 rats. The chamber was replaced by a roller pump (Watson-Marlow Ltd.) which took blood from the proximal aorta and pumped it into the hind limbs. The proximal and distal aortic pressures were approximately equalized before the infusion of ADP from 0.17 to 16.8 μg/min for 30 s. The maximal fall in pressure was recorded. The effect of saline infusion at the fastest infusion rates was also assessed. In 8 rats the effects of repeated doses of ADP were measured by an infusion of 0.84 μg/min for 30 s every 5 min. The aggregation index was calculated from the formula:

$$\text{Aggregation index} = 100\left\{1 - \frac{DP_2 \times PP_1}{PP_2 \times DP_1}\right\}$$

where $PP_1$ is the proximal pressure before and $PP_2$ the maximum pressure during or after ADP infusion.

$DP_1$ is the distal pressure before and $DP_2$ the minimum pressure during or after ADP infusion. Pressures were measured at greatest response.

The results show means and standard errors of the mean with statistical significances calculated by paired or unpaired Student's $t$ test where appropriate. A value for $P$ of $<0.05$ was taken as unlikely to be due to chance.

## Results

A typical dose-response trace to ADP infusion at rates of 0.4 to 8.4 μg/min is given in Figure 2, showing a rise in proximal and a fall in distal aortic pressure. Removal of the filter at maximal aggregation showed white thrombi occluding the pores in those animals in which it was studied. The mean pressure drop across the filter was 3.0 ± 0.7 mmHg in all animals though 3 further rats had pressure drops of 12.7, 8.2 and 7.2 mmHg, before any ADP infusion and were not included in the results. These animals showed a progressive increase in the pressure gradient throughout the experiment and may indicate non-ADP-induced aggregation.

The proximal blood pressure was 67.9 ± 3.8 mmHg in 8 controls and 67.1 ± 3.0 ($P > 0.05$) in 14 test animals before the first dose of ADP. Any animal with a very low blood pressure was rejected even though they all showed quantitatively similar aggre-

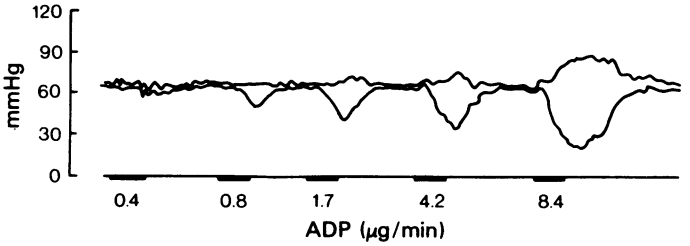


**Figure 2.** Tracing from actual record of pressure changes. Dose-response to increasing adenosine diphosphate (ADP) infusion in a single rat. The infusion was continued for 30 s as shown by the bars. Measurements of pressure in mmHg were taken before ADP infusion and at maximal response.

gation responses. In most animals the blood pressure tended to fall in an experiment and the pressure after both dose-response curves was 60.6 ± 4.8 and 60.6 ± 3.0 in 8 control and 14 test animals respectively.

The effects of vincristine (or a similar volume of saline for the controls) on the dose-response curve to ADP in these 22 animals is shown in Figure 3. The first and second dose-responses in control animals were not significantly different though in many animals there was evidence of reduced aggregation at the same dose of ADP on the second run. In animals treated with vincristine there was a highly significant reduction in aggregation except at 0.2 μg/min ADP. Platelet counts were performed in a further group of 4 control and 6 experimental rats before and immediately after two dose-response curves to ADP. The counts rose from 637,000 ± 120,000 to 905,000 ± 90,000 per $mm^3$ in control animals and 713,000 ± 110,000 to 1,073,900 ± 130,000 in rats given vincristine. Neither of these changes reached statistical significance.

Repeated infusions of ADP at a rate of 0.84 μg/min were performed in 8 animals and confirmed the gradual reduction in aggregation index with repeated infusions (Figure 4). The first infusion produced less aggregation than the second ($P < 0.001$) but thereafter the index gradually fell so that it was significantly less than the maximal response at the seventh infusion ($P < 0.05$). Infusions were performed at 5 min intervals.

ADP-induced peripheral vasodilatation in the hind limbs of the rat was evident by a fall in perfusion

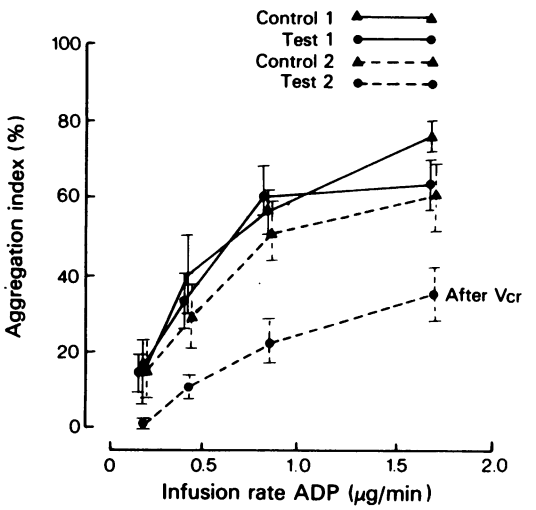


**Figure 3** Stimulus response curves to adenosine diphosphate (ADP) infusion in 14 vincristine treated animals and 8 controls. Values represent means with s.e. means shown by vertical lines. There is no statistical difference between the first dose-response curve to ADP in the control group (▲ ▲) and the test group (● ●) or the second dose-response curve to ADP after intravenous saline (▲ ▲). However, 5 min after intravenous vincristine (Vcr), the dose-response curve (● ●) is significantly depressed except at 0.2 μg/min ADP.

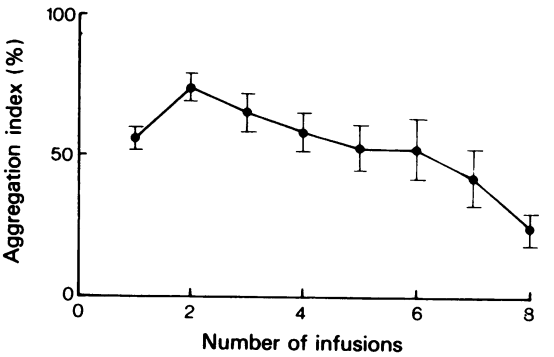


**Figure 4.** The aggregation index from 8 rats after repeated infusions of ADP 0.84 μg/min at 5 min intervals. After an initial rise ($P = < 0.001$) the response declined until $P$ was $< 0.05$ from the initial response at the seventh infusion. Values are means; vertical lines show s.e. means.

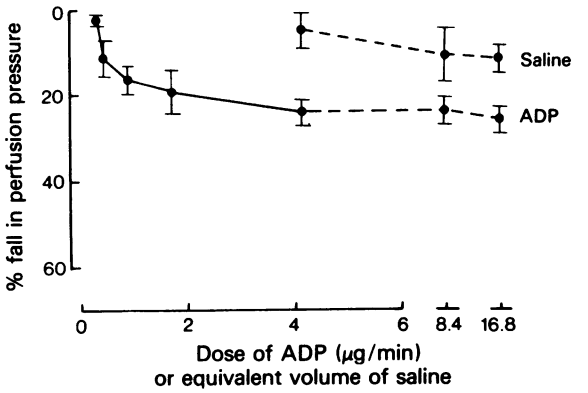


**Figure 5** Vasodilatation in the distal aorta and hind limb of 6 rats produced by adenosine diphosphate (ADP) or saline infusion expressed as the fall in perfusion pressure at constant flow as a percentage of the initial value. Values are mean; vertical lines show s.e. mean.

pressure when the legs were perfused at constant flow (Figure 5). In 6 animals the mean flow to the legs was 3.8 ± 1.0 ml/min with a mean initial pressure of 84.0 ± 6..0 mmHg. Gradually increasing doses of ADP infused over 30 s produced at most a 25% reduction in perfusion pressure at infusion rates of 4.2 μg/min or more. Infusions of similar volumes of saline produced initial rises in perfusion pressure not shown in Figure 5, with subsequent fall in pressure to the values shown in the figure indicating some vasodilatation.

## Discussion

We have shown that vincristine inhibits ADP-induced platelet aggregation using a filter inserted into the aorta of the rat. The method has some advantages over studies performed *in vitro* because the platelets remain within the circulation and are probably exposed to prostacyclin, the most potent anti-aggregatory agent demonstrated so far (Gryglewski, Bunting, Moncada, Flower & Vane, 1976). Prostacyclin is produced by vascular endothelium of both the rat (Villa, Callioni & de Gaetano, 1977; Hornstra, Haddeman & Don, 1978) and human aorta (Moncada, Higgs & Vane, 1977) and has a half-life of approximately 2 to 3 min. The time taken for blood to pass through our system was probably less than 10 s.

Hornstra (1970) showed that there was no difference between his first and second dose-response curves to ADP though he did demonstrate a reduction in aggregation after six doses of ADP with a fall in platelet count. We have confirmed that the aggregation index falls after repeated doses of ADP and this has also been seen by others *in vitro* (O'Brien, 1966; Busfield & Tomich, 1968). In the small number of rats in which it was measured, the platelet count appeared to rise after two dose-response curves to ADP. This effect cannot be due to the thrombocytotic effect of vincristine as it also occurred in the control rats. Born & Philp (1965) suggested that perhaps metabolic breakdown products of ADP might reduce platelet sensitivity to further challenges.

The rise in proximal pressure is probably due to the occlusion of the filter but the fall in the distal pressure may, in part, be due to the vasodilatation induced by ADP. The infusion of saline at high rates also resulted in some vasodilatation which may be because infusions often produced an initial increase in perfusion pressure which may have caused recruitment of more peripheral vessels. The vasodilatation produced by ADP was studied by constant flow perfusion of the hind limbs since ADP infusion will produce changes both in perfusion pressure and flow, complicated by the effects of platelet aggregates as emboli. The concentration of ADP in peripheral vessels will be dependent not only on the infusion rate but also blood flow which will be reduced after platelet impaction at the filter. It is probable that vasodilatation contributed more to the fall in the distal pressure at lower ADP infusion rates (Figure 5) though it is unlikely to have produced such marked depression of aggregation by vincristine at the higher ADP infusion rates studied. It is also unlikely that vincristine is a vasodilator in its own right since there were no changes in proximal pressure after its administration. If vincristine were to affect only ADP-induced vasodilatation, then similar rises in proximal pressure would be seen as before the drug was administered with perhaps a lesser fall in distal pressure. Both proximal and distal pressures were equally reduced suggesting a primary effect on platelet aggregation.

The mean systemic blood pressure was lower than that found by Suggett & Herget (1977) of 88 ± 3.3 mmHg in a similar group of rats not subjected to aortic interference. The volume of the filter chamber and connecting tubing, although small, may be a significant proportion of the rat's circulating blood volume. The satisfactory state of our animals is evident by their maintenance of blood pressure throughout the experiments.

There are difficulties in extrapolating doses of drugs from one species to another (Schmidt-Nielsen, 1970) but we gave a similar dose to our rats per kg body weight to that used in treatment of some forms of leukaemia in man. Although the rat has a higher circulating platelet count than man, the response of platelets to vincristine seems to be similar in both species. The thrombocytotic effect of vincristine has been seen in both (Jackson & Edwards, 1977; Ahn, Byrnes, Harrington, Layer, Smith, Brunskill & Pall, 1978) and we have demonstrated inhibition of platelet

aggregation in our preparation which has also been observed in man (White, 1969).

Vincristine is given as an intravenous bolus in leukaemia where thrombocytopenia is common and bleeding may occur. After vincristine treatment, platelet dysfunction may contribute further to the bleeding tendency and would not be counteracted by the thrombocytotic effects as this depends on normal marrow function (Robertson, Crozier & Woodend, 1970).

Steinherz, Miller, Hilgartner & Schmalzer (1976) have studied patients with acute lymphoblastic leukaemia from one to four weeks after combination chemotherapy including vincristine using *in vitro* tests of platelet aggregation. They found depression of aggregation and suggested that the effects were mediated through the megakaryocyte. The present study would imply that there is also a more direct effect of vincristine on the platelet itself and the contribution of this drug to thrombocytopenic bleeding needs further investigation.

We are grateful for the encouragement provided by Professor J. Richmond and Dr G.R. Barer. E.L. and A.J.S. were supported by the Special Trustees to the former United Sheffield Hospitals. J.F.M. was supported by the Medical Research Council. We are indebted to Mr J. Bamforth for making the chamber.

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(*Received June* 22, 1979.
*Revised August* 18, 1979.)