

Further experience in the use of ancrod (Arvin) to prevent thrombosis on prosthetic heart valves

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Intravenous administration of ancrod produces hypofibrinogenaemia, which may be prolonged by further doses. Hypofibrinogenaemia, maintained for a week, was used to prevent thrombosis on prosthetic heart valves in the calf.

Thirteen calves were used in the study. Three of these were used to determine dose schedules and to test antibody formation. The calf was found to have hyperactive fibrinogenesis, and doses higher than previously reported were required to maintain prolonged hypofibrinogenaemia. Antibodies were detected in the calves treated for prolonged periods and the phenomenon was probably related to the massive dosage required.

In 10 calves, the tricuspid valve was replaced with a polypropylene valve, using cardiopulmonary bypass. Two of these calves, used as controls and not treated with ancrod, showed massive thrombus formation on the valves. The remaining eight calves were treated with ancrod on different dose schedules. In four calves consistent low fibrinogen levels were not achieved. In the other four, treated by a continuous infusion of ancrod, 8–10 units/kg of body weight per day supplemented further by twice daily intravenous injections of 8.0 units/kg, it was possible to maintain sufficiently low fibrinogen levels and to prevent thrombus formation on the valves.

In man, hypofibrinogenaemia is more easily maintained and antibody formation is less likely with the small dosage needed.

Ancrod is a proteolytic enzyme obtained from venom of the Malayan viper *Agkistrodon rhodostoma*. Slow intravenous injection produces hypofibrinogenaemia and the blood becomes incoagulable in man and in the calf (Bell, Pitney, Oakley, and Goodwin, 1968; Singh *et al.*, 1970). Hypofibrinogenaemia may be maintained by further intravenous administration.

In an experimental study Singh *et al.* (1970) exploited the ancrod-sustained hypofibrinogenaemia in the prevention of thrombosis on polypropylene prosthetic heart valves, after replacement of the tricuspid valve in calves. Effective low fibrinogen blood levels were maintained successfully for 72 hours. In the long-term experiments the levels rose in spite of the ancrod administration being continued. It was surmised that the escape of response might be due to species resistance, antibody formation or stimulation of fibrinogen production. The present work was undertaken to elucidate these points and to extend

the period of effective hypofibrinogenaemia to about a week.

Three unoperated calves were studied for dose variation and tested for antibodies after treatment with ancrod for 3 to 21 days.

In 10 calves the tricuspid valve was replaced with a polypropylene disc valve² employing cardiopulmonary bypass. Eight of these were treated with ancrod for seven to nine days on different dose schedules. The remaining two calves were used as controls and paired with two of the calves treated with ancrod. Methods will be described in detail, results reported, and the inferences discussed.

METHODS

EXPERIMENTAL MODEL The tricuspid valve in the calf was replaced with a polypropylene flat disc valve using total cardiopulmonary bypass. Singh *et al.* (1970) and Bonchek and Braunwald (1967) found this model to be satisfactory as, in the absence of anticoagulants, thrombus could be reliably expected to form on the prosthetic valve within 72 hours.

The calf was premedicated with Pethilorfan, 2.0 mg/kg, and anaesthetized by the simple technique reported by Singh, Elliott, and Melrose (1971). The simplicity

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²Manufactured by Portland Plastics, Ltd., Hythe (Kent), England

of the technique consisted in using halothane for induction as well as maintenance of anaesthesia. The different phases of anaesthesia were managed by alterations in the concentration of halothane from 0.5% to 4.0% in a gas mixture of oxygen 70.0% and nitrous oxide 30.0%. Also the safety was enhanced by administering only short-acting drugs, pethidine and methohexitone, in minimal quantities for supplementation.

Ventilation during intrathoracic surgery was maintained by Palmer's 'Ideal Respirator'. Analgesia during the cardiopulmonary bypass was maintained by adding small supplements of pethidine and methohexitone to the perfusate, and the lungs were kept semi-inflated with a mixture of oxygen and air.

Cardiopulmonary bypass was established through a right thoracotomy through the bed of the fifth rib. The calf was heparinized with 3.0 mg of heparin per kg body weight administered intravenously. Both cavae were cannulated through the right atrial appendix for return of the venous blood to the oxygenator, and the aorta was cannulated for the perfusion of oxygenated blood. A disc oxygenator was used in all cases. This was primed with 2.5 to 3.0 litres of bovine blood diluted 50% with a pyrogen-free solution of 5.0% dextrose in water. The oxygenating gas-mixture contained 93.0% oxygen and 7.0% carbon dioxide. Perfusion was accomplished with an occlusive roller pump. A flow rate of 75.0 ml/kg body weight was achieved in all calves.

Right atriotomy was performed using total cardiopulmonary bypass. The tricuspid valve was replaced by the prosthetic valve. The right atriotomy was closed. The bypass was discontinued and the heart and the aorta were decannulated. Heparin was neutralized with an intravenous injection of protamine sulphate, 6.0 mg/kg of body weight. The thoracotomy was closed in layers after achieving haemostasis. Pleural drains were inserted.

The surgical technique of tricuspid valve replacement, pre and postoperative care of the calf, monitoring of the circulatory system, measurement of blood gases, and other supplementary procedures are described elsewhere in detail (Singh *et al.*, 1970).

After completion of the operation, a length of 15-20 cm of a silicone catheter of 2.0 mm internal diameter was inserted in one of the internal jugular veins for administration of ancrod.

TREATMENT WITH ANCROD

Administration All doses of ancrod were administered intravenously into the internal jugular vein, either by venepuncture or through the indwelling silicone catheter.

Induction dose The initial dose was 1.0 unit/kg of body weight in 50 ml of physiological saline given by a constant infusion pump³ over one hour. Subsequent studies showed that the calf can tolerate rapid defibrillation, and induction over a period of one hour is

not necessary. The initial dose was followed by a booster dose of a further 1.0 unit/kg of body weight administered over 5 to 10 minutes, making a total of 2.0 units/kg.

In the previous series (Singh *et al.*, 1970) and in the early experiments in the present series, the induction dose was started six hours after neutralization of heparin with protamine sulphate. However, in four calves, the injection was started one to three hours after the heparin neutralization and no untoward effect was noted.

Maintenance dose Different schedules of maintenance therapy were investigated and the calves were grouped accordingly:

Group I Daily intravenous injection, dosage starting from 2.0 units/kg and increasing to 16.0 units/kg over a period of three weeks; in two unoperated calves.

Group II Continuous infusion of 10.0 units/kg per day by means of a Chronofuser⁴ pump; in one unoperated calf.

Group III Twice daily intravenous injections, each of 8.0 units/kg, increasing to 16.0 units/kg; in two operated calves.

Group IV Continuous infusion of 8.0-10.0 units/kg per day supplemented by twice daily injections, each of 8.0 units/kg; in six operated calves.

FIBRINOGEN ESTIMATION Plasma fibrinogen was measured as thrombin-clottable protein by the method of Ratnoff and Menzie (1951). Venous blood, 4.5 ml, was mixed with 0.5 ml of 3.2% sodium citrate, 0.5 ml of 10.0% epsilon aminocaproic acid (to inhibit fibrinolysis *in vitro*), and 0.1 ml of a 1:10 dilution in saline of antivenene to *Agkistrodon rhodostoma* venom (to prevent action of ancrod *in vitro* in the blood sample). Samples for fibrinogen measurement were routinely collected before the morning injection of ancrod, but further samples were obtained in some calves six hours after the morning injection.

ANTIBODY TEST Three unoperated animals were tested for development of resistance to ancrod by the ancrod resistance test (Pitney, Bray, Holt, and Bolton, 1969). In this procedure, 0.1 unit of ancrod is incubated with 1 ml of plasma. This concentration of ancrod is sufficient to clot⁵ nearly all the fibrinogen in normal human and calf plasma but is insufficient in plasma from patients who have developed antibodies to ancrod. The test was not performed in the operated calves on ancrod treatment, since they were killed while still receiving the drug.

RESULTS

None of the 10 operated calves died. The wounds healed by primary intention. At the end of the

³Manufactured by B. Braun, Melsungen, West Germany

⁴Manufactured by U.S. Catheter and Instrument Corporation, Glens Falls, N.Y., U.S.A.

⁵*In vivo* Ancrod converts fibrinogen into microclot of fibrin which is eliminated from the circulation. The blood, depleted of fibrinogen, becomes incoagulable

experiment, both in the operated and the unoperated calves, thiopentone sodium, 0.5–1.0 g in 10–20 ml of sterile pyogen-free normal saline, was administered intravenously until circulatory arrest occurred. Heparin, 10,000 units, was injected intravenously before the circulatory arrest to prevent post-mortem clotting.

At post-mortem examination the prosthetic valve was examined for thrombus formation. Chambers of the heart, pulmonary artery, and its branches were also examined for thrombosis. The lungs were examined for atelectasis and the thoracic cavity for haemorrhage. Partial collapse of the lung, due to a serous collection of 350 ml in the right pleural cavity, occurred in one calf.

Results in three unoperated and 10 operated calves were grouped according to the dose schedule.

GROUP I In two unoperated calves, following the initial defibrination with 2.0 units/kg, ancrod was administered once daily by intravenous injection, each dose starting at 2.0–4.0 units/kg and increasing to 14.0–16.0 units/kg. Treatment was continued for 20 to 21 days.

Fibrinogen values were estimated on 24-hourly samples withdrawn before the injection in the morning. The values ranged from 55 to 120 mg/100 ml, the mean remaining about 80 mg/100 ml (Fig. 1).

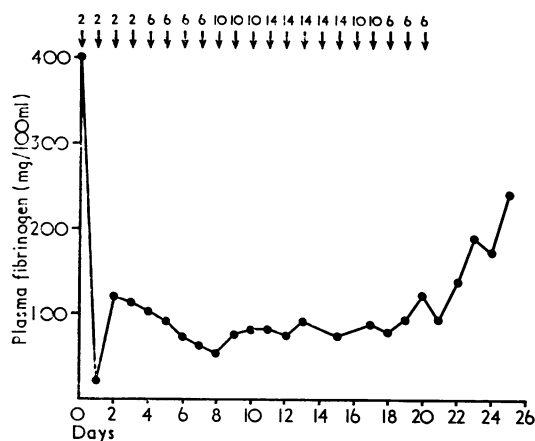


FIG. 1. Twenty-four hourly fibrinogen values in an unoperated calf in group I receiving a single injection of ancrod every day. Figures at the top indicate dose of ancrod in units/kg of body weight. The fall in blood fibrinogen level after the initial defibrination dose was precipitous. The fibrinogen values then rose and ranged between 55 and 120 mg/100 ml, the mean being about 80 mg/100 ml.

Fibrinogen values were also measured in both calves six hours after the injection of ancrod, results of 25 mg/100 ml and 15 mg/100 ml being obtained. It was apparent that the daily injections of ancrod were producing marked hypofibrinogenaemia for some hours, but fibrinogen values increased during the 24-hour interval before the next injection was given.

Antibody to ancrod was detected in the plasma of both calves eight days after the last injection. Total dosage was 170 units/kg in one calf and 234 units/kg in the other.

GROUP II One unoperated calf was given a continuous infusion of ancrod, 10.0 units/kg per day after initial defibrination with 2.0 units/kg. Treatment was continued for three days, and the fibrinogen values ranged from 85 to 110 mg/100 ml.

The ancrod resistance test was negative six days after the last dose of ancrod and the calf responded with hypofibrinogenaemia to an intravenous injection of ancrod, five weeks after the initial treatment. Total dosage was 32 units/kg.

GROUP III Two calves after the defibrinating dose of 2.0 units/kg of ancrod six hours after heparin neutralization with protamine sulphate, were given twice daily intravenous injections, each of 8.0–

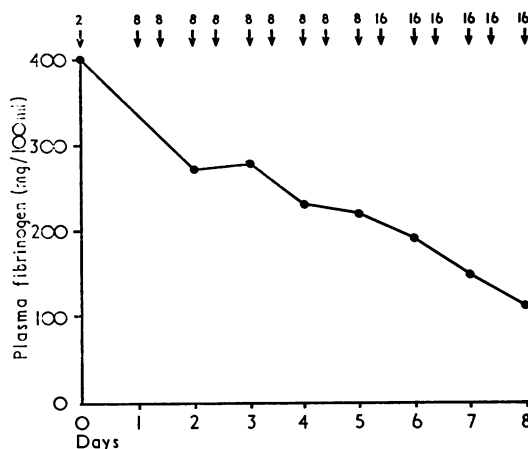


FIG. 2. Twenty-four hourly fibrinogen values in a calf in group III receiving twice daily injections of ancrod after replacement of the tricuspid valve. Doses are shown at the top of the graph. The initial defibrination dose produced slow response. Fibrinogen values fell gradually after increasing the dose of each injection from 8.0 units/kg to 16.0 units/kg, demonstrating that higher doses were required in the operated than in the unoperated calf.

16.0 units/kg. They were killed on the 8th and 9th day after operation. At no time could defibrination be considered satisfactory, in spite of the higher dosage than in the unoperated calves, although the fibrinogen values were slowly falling towards the end of the period (Fig. 2). These animals were more resistant to ancrod than the unoperated ones, presumably because the rate of fibrinogen synthesis was accelerated in the postoperative period. Total dosage of ancrod was 170 and 234 units/kg.

In both these calves, thrombus was present on the atrial and ventricular surfaces of the valve ring. Some thrombus was also present across the orifice of the valve, though not quite so marked as in the untreated calves (*vide infra*).

GROUP IV In six operated calves, ancrod therapy was given by continuous infusion of 8–10 units/kg per day, supplemented by twice daily injections, each of 8.0 units/kg for one week. The last two of the ancrod-treated calves were paired with two more operated calves which, for comparison, were not treated with ancrod.

Fibrinogen values mostly remained about 100 mg/100 ml when measured just before the morning supplemental injection. Values at other times were lower than this. In one calf, fibrinogen levels were estimated six hours after the morning injections on the 5th and 6th postoperative days,

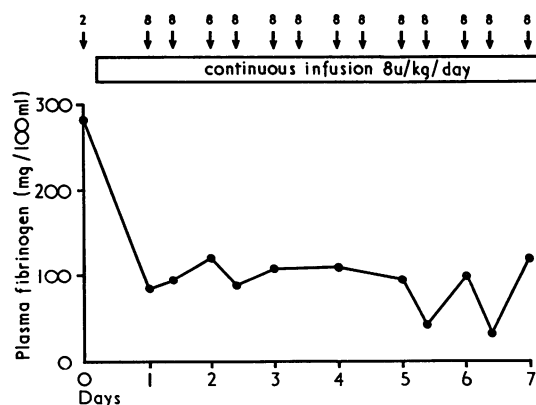


FIG. 3. Twenty-four hourly fibrinogen values in a calf in group IV receiving ancrod after replacement of the tricuspid valve. Ancrod was given by continuous infusion of 8.0 units/kg per day supplemented by twice daily intravenous injections, each of 8.0 units/kg. Values fluctuated around 100 mg/100 ml. However, the values were lower, 45 mg/100 ml and 35 mg/100 ml, when estimated six hours after the injection on the 5th and 6th postoperative day respectively.

and values of 45 mg/100 ml and 35 mg/100 ml respectively were obtained (Fig. 3).

In the two untreated calves, thrombus formation was marked. Thrombus covered both the atrial and ventricular surfaces of the polypropylene valve ring, encroached upon the valve orifice, and covered the valve disc. The sewing ring was heavily covered by the thrombus. The surface of the thrombus was exuberant, shaggy, and irregular (Fig. 4).

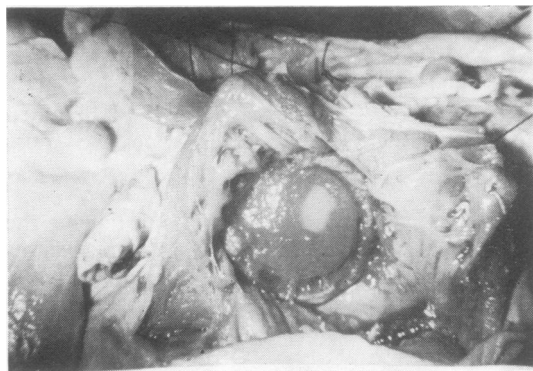


FIG. 4. Shows massive thrombus formation on the prosthetic heart valve in a control calf a week after the valve replacement. The calf did not receive ancrod treatment.

In four of the ancrod-treated calves, both surfaces of the polypropylene ring and the valve orifice were free of thrombus (Fig. 5). The sewing ring was covered by only a thin pannus of fibrin. Its surface was smooth and it was firmly adherent to the fabric.

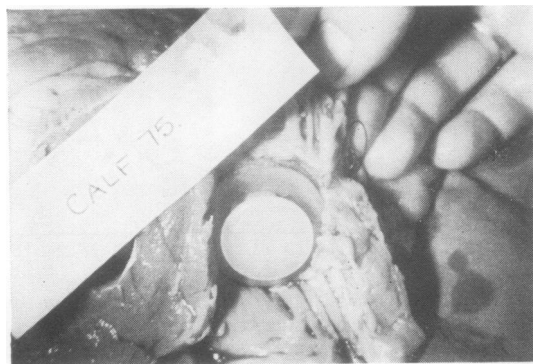


FIG. 5. Shows the valve ring and the orifice free of thrombus formation in a calf in group IV, treated by continuous infusion and supplemental injections of ancrod for one week.

In the remaining two ancrod-treated calves, there was a moderate amount of thrombus formation. In both these calves, the fibrinogen levels had risen during treatment due to mechanical problems with the infusion pump.

DISCUSSION

It is more difficult to maintain low levels of plasma fibrinogen with ancrod in the calf than in man. Maintenance therapy of 1 unit/kg every 12 hours in man results in steady plasma fibrinogen values of about 50 mg/100 ml (Bell *et al.*, 1968), and even a single 24-hourly injection of 1–2 units/kg is usually effective (Pitney, 1970). In the unoperated calves, fibrinogen values 24 hours after doses of 14–16 units/kg were usually about 100 mg/100 ml, although they were much lower than this 6 hours after the injection. The calf is, therefore, sensitive to ancrod but the effect of each injection is more transient than in man. The most likely explanation is that the rate of fibrinogen synthesis is high in calves receiving ancrod.

It was even more difficult to maintain hypofibrinogenaemia in the operated calves, presumably because the stress of operation stimulated fibrinogen synthesis further. The final dose scheme adopted was a continuous infusion of 8–10 units/kg per day, supplemented by twice daily injections, each of 8.0 units/kg. This schedule resulted in maximal plasma fibrinogen values of about 100 mg/100 ml just before the morning booster injection. Values were lower than this at other times during the 24-hour period (Fig. 3). It may, however, be possible to achieve even lower fibrinogen blood levels by further manipulation of the maintenance dose schedule.

Two calves given repeated ancrod injections for 20–21 days developed antibodies, whereas an animal treated for only three days did not. Antibody production is likely to be related to the length of therapy and the quantity of dosage. This may effectively prevent long-term ancrod treatment in the calf. The position with regard to man is not yet known; but resistance has developed in patients given ancrod intravenously for prolonged periods (Pitney *et al.*, 1969). As high doses are not required in man, total dosage will remain

small for short periods of treatment and may account for the resistance developing only after prolonged treatment.

The valves remained free of thrombus in the four calves in which it was possible to maintain adequate hypofibrinogenaemia. Thus it would seem tempting to treat patients with ancrod in clinical practice in the early postoperative period after valve replacements, until oral anticoagulants became effective. However, in man, fibrinogenesis is not so active as in the calf and much smaller doses produce adequate hypofibrinogenaemia for prolonged periods (Bell *et al.*, 1968; Pitney, 1970). Therefore, considerable caution will have to be exercised in using ancrod in man, because of the increased risk of haemorrhage in the early postoperative period.

The project was supported and ancrod supplied by Twyford Laboratories, Ltd., London N.W.10.

We should like to thank Professor J. F. Goodwin and Professor H. H. Bental for constant encouragement to continue this study; Mr. L. Wosornu, F.R.C.S., and Miss S. P. Allwork for assistance in surgery; Mrs. J. Becket, Messrs E. Williams, S. Adams and R. L. Elliott for technical assistance; Messrs. J. Robson, D. Wilson, and B. Verney for care of the calves; Mr. K. Ganeshaguru for assistance in the haematological studies; Miss G. Bolton for preparing the graphs; and Miss S. Rankine for secretarial assistance.

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