

The use of ancrod to prevent thrombosis on prosthetic heart valves

An experimental study

M. P. SINGH, H. H. BENTALL, W. R. BELL,
E. G. J. OLSEN, and S. P. ALLWORK

Royal Postgraduate Medical School of London

Ancrod (Arvin) on slow intravenous administration converts fibrinogen into fibrin at a rate which does not cause intravascular thrombosis. The fibrin is eliminated from the circulation and the blood is thus rendered incoagulable. To test the efficacy of ancrod in the prevention of thrombosis after prosthetic replacement of the heart valves, the tricuspid valve was replaced with a polypropylene mitral valve in 17 calves, using cardiopulmonary bypass. Five calves were eliminated from the study. Eleven calves were treated with ancrod, and one untreated calf was used as a control. Four out of five prosthetic valves in calves treated with ancrod for up to 72 hours were free of thrombus formation, and one out of three at one week. In the long-term study, fibrinogen titres rose after varying intervals, in spite of continual treatment with ancrod. This 'escape' of fibrinogen titres from the control of ancrod may be due to species resistance or to the development of immunity. Further study is in progress to elucidate this point and to gain further knowledge for the clinical application of ancrod.

Ancrod (Arvin) is a proteolytic enzyme derived from the venom of the Malayan pit viper (*Agkistrodon rhodostoma*). On slow intravenous administration it converts the circulating fibrinogen into fibrin micro-clots throughout the vascular compartment. The fibrin is then removed by fibrinolysis and phagocytosis. After an adequate dose in man the level of fibrinogen falls to below 50 mg.%. Repeated doses are required to maintain hypofibrinogenaemia.

This experimental study was undertaken to determine the role of this new 'anticoagulant' in the prevention of thrombus formation on prosthetic heart valves.

Prosthetic valves, designed for mitral replacement in man, are vulnerable to thrombus formation when used experimentally in calves in the tricuspid area. Bonchek and Braunwald (1967) observed in calves that, after replacement of the tricuspid valve with a polypropylene mitral valve¹, thrombus formation occurred within 72 hours unless measures were taken to prevent it. We used this technique as a model for testing the effect of ancrod. Thrombus formation was predictable and the results could be compared

with the controls which they had already established.

In 17 calves the tricuspid valve was replaced with a polypropylene mitral valve using cardiopulmonary bypass. Three calves did not survive the operation and two others died in the post-operative period. One calf was not tested and was used as a control. Eleven calves were treated with ancrod over periods of 36 hours to 29 days.

TECHNIQUE

Young Hereford bull-calves were reared until they could eat and drink a normal diet by themselves before the operation. The average age at operation was 7 weeks and the average weight was 49.2 kg.

Administration of antibiotics, penicillin 1,000,000 units and streptomycin 0.5 g., by intramuscular injection twice a day was started 48 hours before the operation.

The calves were premedicated with Pethilorfan, 2.0 mg. per kg. of body weight. Anaesthesia was induced with a mixture of 70% nitrous oxide and 30% oxygen. Halothane (B.P.) 0.5% was added and gradually increased to 4.0%. Anaesthesia was maintained with oxygen and halothane, 1.5 to 3.0%, administered through an endotracheal tube. Ventilation was maintained with the Palmer Ideal Respirator.

¹This valve was manufactured by Portex Plastics Ltd.

Continuous gastro-intestinal decompression was begun.

Arterial and central venous pressures and an electrocardiogram were monitored throughout the operation.

The standard low-prime Rygg bag oxygenator was primed with heparinized bovine blood and diluted 50.0% with 5.0% dextrose solution. Two oxygenators were used if the weight of the calf exceeded 50.0 kg. The perfusate was oxygenated with a mixture of 93.0% oxygen and 7.0% carbon dioxide. A DeBakey type roller pump was used for perfusion and a perfusion rate of 75.0 ml./kg. of body weight was obtained in all the experiments.

Samples of 2.0 ml. of venous blood were withdrawn from the calf and mixed with sequestrene. Haemoglobin and haematocrit values were estimated and platelet counts were done. Repeated estimations of the pH, oxygen, and carbon dioxide gas tensions of the perfusate were done before and during the bypass procedure and suitable corrections were made.

OPERATIVE PROCEDURE

A right thoracotomy was performed with the calf in the left lateral position. The chest was entered through the bed of the fifth rib. The right atrium was exposed by incising the pericardium longitudinally, anterior to the right phrenic nerve.

Heparin, 3,000 units/kg. of body weight, was administered intravenously. The two cavae were cannulated through separate incisions in the right atrium. The aorta was cannulated for return of the oxygenated blood. These cannulae were connected to the appropriate limbs of the heart-lung circuit for the cardiopulmonary bypass. The perfusion was started. Hypothermia was not used, nor was the heart fibrillated.

The right atrium was opened during complete cardiopulmonary bypass. The tricuspid valve was excised and a sterilized polypropylene mitral prosthesis was inserted with 2/0 mersilene interrupted sutures. The maximum flow outlet of the prosthetic valve was directed towards the outflow tract of the right ventricle.

The atrial incision was closed. The cardiopulmonary bypass was discontinued. The heart was decannulated when the cardiac function was sustained satisfactorily. The pericardium was then closed loosely with interrupted sutures. Protamine sulphate, 6.0 mg./kg. of body weight, was administered intravenously. Two intercostal drains were inserted and connected to a chest drainage set. Haemostasis was secured and the chest was closed in layers. Any oozing points in the edges of the skin were carefully secured.

The internal jugular vein was cannulated for administration of ancrod and withdrawal of blood samples with a vinyl catheter, 25.0 cm. in length and 2.0 mm. in diameter.

Anaesthesia was discontinued and the calf was gradually allowed to breathe spontaneously.

POST-OPERATIVE CARE

A recovery room was specially prepared. The calves were nursed in the standing position in a specially designed canvas sling, suspended from a Pavlov frame. The animals seemed more comfortable standing up and, moreover, their respiratory movements were not restricted, the chest tubes drained better, and the pressure monitor lines were not compressed by the calf lying on them inadvertently.

Blood transfusion was continued to replace the blood lost from the pleural drains. Bleeding was usually minimal after six to eight hours. Intravenous infusion of sterile pyrogen free 0.18% sodium chloride in 4.3% dextrose was continued until the calf was able to drink normally.

Monitoring of the arterial and central venous pressures and the electrocardiogram was continued. Repeated biochemical, haematological, and gas tension estimations were made and appropriate corrections were instituted.

The calves were usually well enough to be transferred to their pens within 24 hours. The pleural drains, pressure monitoring lines, and the electrocardiogram electrodes were removed before the transfer.

Rectal temperature was recorded twice a day.

Antibiotics were administered twice a day in dosages as started pre-operatively and were continued for a week or longer, if indicated.

TREATMENT WITH ANCROD

DOSE Ancrod was administered intravenously in a dose of one ancrod unit per kilogram of body weight. The dose was increased, to a maximum of 1.5 unit/kg., if the fibrinogen titre (*vide infra*) increased.

METHOD The first dose was given six hours after termination of the cardiopulmonary bypass. Defibrination was induced slowly to prevent intravascular deposition of fibrin. The dose was mixed with 100 ml. of sterile normal saline solution and administered slowly with a constant infusion pump over a period of one hour. At the conclusion of this, a further dose of the same quantity was administered intravenously in 10 ml. of sterile normal saline, with a syringe, over a period of 10 minutes. Subsequent doses were administered eight-hourly. The method depended on the time of administration. During the night the dose was administered by a constant infusion pump which started by clockwork at a pre-set time. During the daytime the doses were administered with a syringe.

CONTROL OF THERAPY The plasma fibrinogen titre was estimated daily using a modification of

the method described by Sharp, Howie, Biggs, and Methuen (1958). During the early part of the study, 2.0 ml. of venous blood was collected in bottles containing sequestrene (1 mg. per ml. of blood). It was, however, observed that the presence of excess ancrod in the blood sample interfered with the test by producing fibrin clots *in vitro*. Therefore, in the later experiments, the samples were taken into bottles containing a solution of sodium citrate, epsilon aminocaproic acid, and antivenene, described by Bell, Bolton, and Pitney (1968). Blood, 4.5 ml., was added to 1.1 ml. of the mixture.

Bovine thrombin was added to serial dilutions of the plasma of the calf under test. The highest dilution in which a fibrin clot was observed was recorded as the fibrinogen titre. The titre before starting the ancrod treatment was usually 1:512.

With the dose schedule of ancrod which we used, the plasma samples 24 hours after commencement of the therapy were incoagulable with thrombin. Thereafter the fibrinogen titre ranged from zero to 1:32 in the calves treated for five days or less.

In three calves (nos. 45, 49, and 55; Table) given extended courses of treatment, an 'escape phenomenon' was observed, which occurred on the 6th, 22nd, and 18th day respectively. Despite the continued administration of ancrod, their fibrinogen titres rose from zero to 1:32, 1:128, and 1:512, and remained at those levels.

After these observations in the earlier experiments, the dose of ancrod was increased if there was any tendency for the titre to rise. However, in calves 52 and 53 the fibrinogen titres could not be estimated accurately in some of the samples

because of the presence of excess ancrod. The blood samples were subsequently collected in special bottles (*vide supra*) to eliminate this error.

On one occasion (in calf 56) the infusion pump failed to deliver the full dose of ancrod, and the control of fibrinogen level was lost.

END OF EXPERIMENT

Except in the control experiment, or when the calf died of some other cause, the experiment was terminated by intravenous administration of 0.5-1.0 g. of thiopentone sodium in 10 ml. of normal saline. Heparin, 10,000 i.u., was injected intravenously before the circulatory arrest to prevent post-mortem clot formation.

A complete post-mortem examination was made. The wounds were observed for healing and the thoracic cavity for evidence of haemorrhage. The prosthetic valve, all the chambers of the heart, and the pulmonary artery and its branches were examined for the presence of thrombus formation. Thrombus, when present, was examined microscopically, the preparations being stained with haematoxylin and eosin and van Gieson stains.

RESULTS

Neither wound disruption nor excessive haemorrhage was observed in any of the calves. Of the 17 calves operated upon, three did not survive the operation. Two calves died early in the post-operative period, one six hours after operation before ancrod treatment was begun and the other after eight hours, having received only the initial

TABLE

RESULTS OF TREATMENT WITH ANCROD AFTER REPLACEMENT OF THE TRICUSPID VALVE IN CALVES

Period of Study	Calf No.	Duration of Experiment	Control with Ancrod Treatment	Thrombus Formation			
				Valve Orifice	Atrial Surface of Valve Ring	Ventricular Surface of Valve Ring	Propagation of Thrombus
Control	44	16 hours	Not given	+	+	+	+
Long-term Study (3 weeks or more)	45	29 days	Escape	±	+	+	0
	49	27 days	Escape	±	+	+	0
	51	21 days	Escape	±	+	+	0
Intermediate Study (up to 7 days)	48	7 days	Adequate	0	±	±	0
	52	7 days	Insufficient data	0	+	+	0
	53	7 days	Insufficient data	0	+	+	0
Short-term Study (up to 72 hours)	55	36 hours	Adequate	0	0	0	0
	57	72 hours	Adequate	0	0	0	0
	59	72 hours	Adequate	0	0	0	0
	60	72 hours	Adequate	0	0	0	0
	56	72 hours	Doubtful	0	+	±	0

0, Free of thrombus, ±, Pannus formation, +, Thrombus formation.

dose of ancrod. No thrombus was present in either of these animals and their data have been excluded as their survival was too short for analysis. The remaining animals were divided into four groups (Table).

CONTROL EXPERIMENT Ancrod was not given to calf 44, which died 16 hours after operation. Marked antemortem thrombus was present on both the atrial and the ventricular aspects of the valve ring and was continuous with that within the valve orifice; the thrombus was propagated along the outflow tract of the right ventricle into the pulmonary artery. The mechanism of both the pulmonary and prosthetic valves was completely enmeshed in thrombus. Bonchek and Braunwald (1967) observed thrombus formation within 72 hours in three controls using the same technique.

LONG-TERM STUDY There were three calves in this group (nos. 45, 49, and 51) and all were treated with ancrod. One calf (no. 51) died three weeks after operation from infection of the inguinal wound. The experiments on the other two were terminated on the 27th and 29th day respectively. All these calves showed 'escape phenomenon' on the ancrod treatment.

Thrombus had formed in concentric layers on the atrial and ventricular aspects of the valve ring but there was no thrombus within the valve. The thrombus was not propagated. Small emboli were detected in the peripheral lung fields of all the animals in this group.

SURVIVAL FOR ONE WEEK Three calves (nos. 48, 52, and 53) were killed after one week of treatment with ancrod. In one calf (no. 48) there was only a thin pannus of platelet thrombus on both aspects of the valve ring. The valve orifice was free and the thrombus was not propagated.

In the other two calves, thrombus formation was more marked, but again there was no thrombus within the valve orifice nor was it propagated.

The excess of ancrod present in the blood samples interfered with the estimation of fibrinogen titres in the two later calves, and the results could not be correlated with the titres.

SURVIVAL UP TO SEVENTY-TWO HOURS No thrombus formation was observed in four out of the five calves (nos. 55, 57, 59, and 60). In the fifth calf (no. 56) one dose of ancrod was missed owing to mechanical failure of the infusion pump. In this calf thrombus was present on the atrial

aspect of the valve ring only; the rest of the valve was free of thrombus.

DISCUSSION

With the experimental model in our series, massive propagated thrombus formed in the untreated calf within 16 hours of operation, which agrees with the findings of Bonchek and Braunwald (1967), who observed thrombus formation on the prosthetic valves within 72 hours in all untreated calves in a similar experimental model. No thrombus was seen in a calf which died six hours post-operatively. Treatment with ancrod was therefore begun within six hours after the operation.

After treatment with ancrod, in our series, four out of five calves (80%) were free of thrombus formation at 72 hours and one out of three (33%) at one week. When thrombus did form while the calves were treated with ancrod it was almost limited to the ring of the prosthetic valve. There was no thrombus in the atrium or ventricle. The orifice of the valve was free, and the thrombus was not propagated, whereas in the untreated (control) calf the mechanism of the valve was completely enmeshed in thrombus which was propagated into the right ventricle and the pulmonary artery.

Thrombus formation, in the long-term study, could be related to an 'escape phenomenon' and consequent rise in the fibrinogen titres despite continual treatment with ancrod. The 'escape phenomenon' might be due either to species resistance or to development of immunity, and merits further study. The presence of infection in the inguinal wound in one of the calves might have been a contributing factor by accelerating the formation of fibrinogen.

It was feared that uncontrollable haemorrhage might occur and wound healing might be slow, owing to the total defibrination with ancrod, in spite of maximum haemostasis having been achieved both inside the chest and in the wound. However, excessive haemorrhage or wound disruption did not occur after early administration of ancrod in the calves. This does not, however, rule out the possibility of a risk of haemorrhage in man because of the species differences. More knowledge will have to be gained before a clinical trial is carried out.

We should like to thank Professor D. G. Melrose for his guidance at every stage of the study and for

providing the experimental facilities at the Nuffield Unit of Clinical Physiology; Dr. W. R. Pitney for supervising the haematological studies; Mrs. J. Becket, Miss G. Bolton, Messrs. R. Elliott, S. Adams, E. Williams, and R. Florio for their technical assistance; Mr. D. Wilson and Mr. J. Robson and their staff for the care of the animals; and Miss B. Allen, Mrs. L. Goodhart, and Miss S. Taylor for typing the paper. The project was supported by a grant from the Twyford Laboratories Ltd. Dr. W. R. Bell was in receipt of U.S. Public Health Service Fellowship Award No. 1-F2-HE-35,260-01. Dr. E. G. J. Olsen

was in receipt of a grant from the Medical Research Council for research on ancrod.

Ancrod was supplied by Twyford Laboratories, Ltd., London, N.W.10.

REFERENCES

- Bell, W. R., Bolton, G., and Pitney, W. R. (1968). The effect of Arvin on blood coagulation factors. *Brit. J. Haemat.*, **15**, 589.
- Bonchek, L. I., and Braunwald, N. S. (1967). Modification of thrombus formation on prosthetic heart valves by the administration of low molecular weight dextran. *Ann. Surg.*, **165**, 200.
- Sharp, A. A., Howie, B., Biggs, R., and Methuen, D. T. (1958). Defibrination syndrome in pregnancy: value of various diagnostic tests. *Lancet*, **2**, 1309.