Therapeutic Defibrination and Heparin Therapy in the Prevention and Resolution of Experimental Venous Thrombosis


SUMMARY

The application of phenol to the femoral veins of anesthetized dogs resulted in an occluding thrombus in 24 of 25 veins at 1 week. Serial venography and eventual histology showed that these veins remained occluded over a 5-week observation period, the thrombi undergoing organization. Therapeutic defibrination was achieved with administration of an enzyme from venom of the pit viper (Arvin). Arvin, administered for 1 week immediately following phenol application and before thrombus formation had occurred, prevented thrombosis in all 10 such veins, and serial venography for a further 4 weeks showed that the veins remained patent. Administration of Arvin was begun 24 hours postoperatively, when venography had demonstrated an occluding thrombus and failed to clear any of 11 veins after 3 weeks of treatment.

Intravenous administration of heparin, 10,000 units, at 6, 6, and 12-hour intervals in a 24-hour period, was begun immediately after phenol application and continued for 1 week; 10 of 12 veins were blocked at 1 week. Following the same dose of heparin every 6 hours four of eight veins were blocked at 1 week.

The results indicate that when the vascular endothelium is damaged, therapeutic defibrination is more effective than heparin therapy in preventing venous thrombosis. Arvin therapy has no significant thrombolytic effect even if the thrombus is less than 24 hours old.

Additional Indexing Words:
Arvin Anticoagulants Phenol Venography Thrombolysis

The bite of the Malayan pit viper (Agkistrodon rhodostoma) results in the development of severe hypofibrinogenemia and the blood may become incoagulable. Arvin is a proteolytic enzyme purified from the venom of the Malayan pit viper. It converts plasma fibrinogen into fibrin microclots which are removed from the circulation both by fibrinolysis and through phagocytosis by cells of the reticuloendothelial system. Profound hypofibrinogenemia may be achieved and maintained by repeated injections of Arvin, without a significant reduction of other coagulation factors.

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This work was supported in part by a grant from Twyford Laboratories, London, England. Dr. Olsen is in receipt of a grant from the Medical Research Council. Dr. Rahimtoola was initially in receipt of scholarship from Mayo Clinic and later from Royal Postgraduate Medical School for part of this study.

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Received May 11, 1970; revision accepted for publication June 22, 1970.
Therapeutic defibrination, achieved by repeated injections of Arvin, has been demonstrated to be clinically safe. Initial reports of its use in patients with venous thrombosis have suggested that it may cause lysis of venous thrombi. During therapeutic defibrination, changes have been observed in the blood-fibrinolytic system; however, these changes are secondary to intravascular fibrin deposition and are not helpful in the lysis of preformed thrombi. Therefore, it is difficult to see how Arvin therapy could have contributed to the lysis of peripheral venous thrombi.

An experimental model of venous thrombosis would be useful in assessing the role of therapeutic defibrination. We have produced experimental venous thrombi in dogs by chemical damage to the vascular endothelium and have studied the natural progression of such thrombi. The role of therapeutic defibrination and of heparin therapy in the prevention and lysis of these venous thrombi has been assessed.

Methods

The Experimental Model

The procedure used is similar to that described by Marshall and associates for the production of a thrombus in the inferior vena cava. Mongrel dogs weighing approximately 10 kg were anesthetized with intravenous thiopental and, following intubation, anesthesia was maintained by nitrous oxide, oxygen, and halothane. The femoral vein on one or both sides was exposed by an incision about 4 to 6 cm long in the groin and dissected free for a length of about 2.5 to 4 cm. A fine polyethylene tube was introduced into the lumen of the vein via a tributary, all other tributaries in the area being ligated (fig. 1). A segment of femoral vein was emptied of blood and isolated between two bulldog clips. A solution of 80% phenol was injected through the polyethylene tube to produce distention of the isolated femoral vein segment. The phenol was allowed to remain in the vein for approximately 90 sec; it was then aspirated through the polyethylene tubing, and the vein flushed with saline. The tubing was then removed from the vein, the tributary ligated, the bulldog clips were removed, and blood flow was restored. The wound was closed with subcutaneous catgut sutures followed by nylon mattress sutures in the skin. The length of time that phenol is left in the vein is important. If the time is too short (45 to 60 sec), the incidence of thrombosis is significantly reduced.

Some animals were used for two different experimental procedures, the veins on each side being operated on at different times. To avoid confusion in the presentation of results, assessment is based on the number of veins, and not the number of dogs, in each experimental group. At autopsy, the pulmonary arteries were not examined for pulmonary emboli.

Assessment of Thrombosis

Intravenous venography was performed under pentothal anesthesia using 20 ml of 60% Urografin* (a mixture of sodium and methylglucamine salts of diatrizoate) as contrast material. Injection was made into a vein in the dorsum of the foot and a single exposure taken at the completion of the injection. Initially, cineangiograms were also performed, and there was complete correlation with the single exposure film. Venograms were performed at intervals of up to 5 weeks following application of phenol to the vein. Only complete obstruction of the main femoral vein was accepted as evidence of thrombosis. Femoral veins were examined histologically following staining of paraffin-embedded sections with hematoxylin and eosin, Martius scarlet blue and Weigert's elastic van Gieson.

Arvin Therapy

Arvin‡ is a polypeptide with proteolytic

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*Urografin, Schering AG Berlin, Germany.
EXPERIMENTAL VENOUS THROMBOSIS

activity purified from the venom of the Malayan pit viper. It is supplied in 1.0 ml sterile ampules containing approximately 75 units (one unit containing 2 µg of the active agent) dissolved in a sodium phosphate-sodium chloride buffer at pH 7.0. One unit of Arvin will clot a solution of human fibrinogen in the same time as does one N.I.H. (National Institutes of Health) unit of thrombin at 37 C.

Defibrination was achieved by intravenous infusion of one unit of Arvin per kilogram of body weight. The dose was made up in 25 ml of physiologic saline and infused over 1 hour. Defibrination was maintained by intravenous injection of one unit per kilogram every 24 to 48 hours, the timing of injections being dependent upon the plasma fibrinogen concentration. The aim of therapy was to maintain plasma fibrinogen, estimated as thrombin-clottable protein, below 50 mg/100 ml. The duration of therapy was 1 week for prophylactic treatment and 3 weeks for treatment of established thrombosis.

Heparin Therapy

Heparin was administered by intermittent injection through an indwelling catheter introduced via the jugular vein to the central circulation. Heparin in dosages of 10,000 units was given either three or four times in a 24-hour period. In one schedule, heparin was given at 7 a.m., 1 p.m., and 7 p.m., and in the other schedule, heparin was given every six hours. Heparin therapy was continued for 1 week and was monitored by measurements of whole blood coagulation time and assay of plasma heparin concentration. Coagulation times and plasma heparin concentrations were determined 6 hours after the injection of 10,000 units of heparin; that is, immediately prior to the next dose of heparin. Coagulation times were prolonged to two to five times the normal value and plasma heparin concentrations were usually 0.5 to 2.0 units/ml. Thus, the animals receiving heparin by the 6-hourly schedule had a high level of anticoagulation at all times.

Figure 2

Venograms in a dog immediately after application of phenol to the femoral vein (left panel) and 24 hours later (right panel).

Circulation, Volume XLII, October 1970
Serial venograms following application of phenol to femoral vein. (A) Immediately after completion of operation, showing irregular thinning of the vein, which is, however, still patent. (B) One week later, showing complete occlusion of the vein with a prominent collateral circulation. The arrow points to the lower end of the occlusion, but the upper end is not visualized. (C) Three weeks after operation. (D) Five weeks after operation; the appearances are similar to those at 1 week.

Results

Control Experiments

Venograms performed immediately after closure of the groin wounds showed the femoral veins to be patent, but occlusion with thrombus was observed on subsequent examinations. The animals suffered no outward ill effects from the femoral vein thrombosis; that is, there was no edema or increase in limb width. Collateral pathways were opened up (even within 24 hours) and flow effectively bypassed the obstructed vessel (fig. 2).

In dogs surviving for 1 week after the operation, 24 of 25 veins treated with phenol had become occluded with thrombus. Serial venograms showed that an occluded vein did not undergo spontaneous thrombolysis or recanalization during a 5-week period of observation (table 1). The number of veins studied decreased as the weeks progressed for animals were sacrificed during the study for histologic examination of the veins. All surviving animals were examined by venography. An illustrative series of venograms is shown in figure 3.

Histologic study of thrombosed veins at various times after phenol application showed complete correlation with venography. Histologic examination of the vein 24 hours after phenol application showed that the thrombus consisted of masses of platelets and fibrin with a small admixture of leukocytes and red cells and was closely adherent to the vessel wall;

<table>
<thead>
<tr>
<th>Time after phenol application (wk)</th>
<th>Veins (no.)</th>
<th>Thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
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<td>6</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 1 Results of Application of Phenol to the Femoral Vein in the Control Animals


Table 2

<table>
<thead>
<tr>
<th>Time after phenol application (wk)</th>
<th>Veins (no.)</th>
<th>No. Thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
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</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Results When Arvin Therapy Was Commenced Immediately after Application of Phenol to the Femoral Vein

The endothelium of the vein wall showed damage (fig. 4). In some specimens, the media of the vein also showed small areas of necrosis. Veins examined 1 week after the application of phenol showed spindle-shaped cells invading the thrombus and by the end of the second week, organization was well advanced with the appearance of collagen and clefts lined by endothelial-like cells. Collagen tissue continued to increase and, by 5 weeks, the organized thrombus consisted almost entirely of collagen in which a few small capillary spaces were present (fig. 5).

Prophylactic Treatment with Arvin

In 10 dogs, immediately after closure of the operation wounds, venography was performed to assure patency of the veins and Arvin therapy was started. Venography was repeated after therapeutic defibrination was achieved to ensure patency of the veins. Plasma fibrinogen values were maintained below 50 mg/100 ml by injections of Arvin every 24 to 48 hours. Severe hemorrhage from the wounds proved fatal in three dogs, 1 to 3 days after administration of Arvin was begun.

Figure 4

Histologic section of thrombosed vein 24 hours after application of phenol. Note the absence of reaction between the thrombus and the vessel wall. The space within the lumen of the vessel is an artifact (Weigert's elastic van Gieson stain; reduced from × 20).

Figure 5

Histologic section of thrombosed vein 5 weeks after application of phenol. The original lumen is entirely occluded by fairly dense collagen. The darker staining area within the collagen is a recent thrombus in a newly developed vascular channel. Dense collagen surrounds the vessel wall. (Weigert's elastic van Gieson stain; reduced from × 20).

Figure 6

Histologic section of vein from dog which had received Arvin for 1 week following phenol application. The lumen is patent and the vessel wall shows no significant abnormality (Weigert's elastic van Gieson stain; reduced from × 20).
Figure 7

Serial venograms in a dog which had received Arvin for 1 week following phenol application. (A) Immediately after induction of defibrination. (B) One week later. (C) Five weeks after application of phenol, the vessel has remained patent.

Table 3

Results of Prophylactic Anticoagulant Therapy for 1 Week

<table>
<thead>
<tr>
<th>No. of dogs</th>
<th>Treatment</th>
<th>Animal deaths during experimental period</th>
<th>No. of veins completing treatment</th>
<th>No. of veins blocked</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>None</td>
<td>1 – Distemper</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>10</td>
<td>Arvin</td>
<td>3 – Bleeding</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Heparin 10,000 units; 7 a.m.; 1 &amp; 7 p.m.</td>
<td>2 – Bleeding*</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>Heparin 10,000 units 6 hourly</td>
<td>4 – Bleeding</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

*Two animals were dropped from the study because of blocked catheters.

The agent was continued in the seven remaining animals for a total period of 1 week, and all the veins (10) were then examined by venography. All 10 veins were found to be patent (table 2). Two veins were examined histologically at this time and were found to be free of thrombus. The vascular endothelium had regenerated and, apart from...
a few inflammatory cells in and around the vessel wall, the appearances were indistinguishable from normal (fig. 6). The remaining veins were examined radiologically and histologically for 5 weeks after phenol application, in other words, 4 weeks after Arvin therapy was discontinued, and were found to be free of thrombus (table 2). Serial venograms of one such vein are shown in figure 7.

### Prophylactic Treatment with Heparin

Heparin was administered to 10 dogs immediately after the operation in a dose of 10,000 units at intervals of 6, 6, and 12 hours during any 24-hour period. Two animals died of bleeding from the site of the operative wounds, and treatment was discontinued in two other animals because of technical difficulty with the indwelling catheter. There were 12 veins available for examination at the end of 1 week of heparin therapy; venography and histology revealed that 10 of these 12 veins were occluded by thrombus (table 3). The incidence of thrombosis was not significantly different from that in the control animals ($P > 0.3$). The $P$ value was obtained by applying a standard chi-square test with Yates correction.

A further eight dogs were given heparin in a dose of 10,000 units every 6 hours. The more frequent injections (the 6-hourly schedule) were associated with a high incidence of bleeding, which proved fatal in four of the eight dogs. Of the eight veins available for study at the end of 1 week of heparin therapy, four were occluded by thrombus (table 3). The incidence of thrombosis was significantly less than in animals given heparin three times a day or in the control animals ($P < 0.01$), but was greater than that seen in the animals treated with Arvin ($P < 0.05$).

Thrombi which occurred during heparin therapy showed some differences from those in control animals when examined histologically 1 week after the application of phenol. Organization had not commenced at 1 week in thrombi from the heparin-treated dogs, whereas it was always present in thrombi obtained from control animals. The appearances suggested that the thrombi in the treated animals were more recent than those in the controls.

### Treatment of Established Thrombosis with Arvin

Arvin treatment was commenced in nine dogs 24 hours after operation when venography had shown that 13 of 18 veins subjected to phenol application were obstructed. Thus, the thrombus in these cases was less than 24 hours old. Hemorrhage from groin wounds proved fatal in two dogs within a few days of commencing Arvin therapy. Therapy was continued in the seven remaining dogs for 3 weeks, at the end of which time venography was repeated and the veins were examined histologically. None of the thrombosed veins showed restoration of vein patency during Arvin therapy (table 4). Histologic examination showed that organization was proceeding in the thrombi of the Arvin-treated animals but lagged behind that present in the control animals at an identical stage.

### Treatment of Established Thrombosis with Heparin

Large doses of heparin (I.V. 10,000 units every 6 hours in animals weighing about 10 kg) were not successful in preventing thrombosis. This dosage of heparin was associated with a high mortality and morbidity of the animals, and thus heparin was not used in the treatment of established thrombosis.

### Discussion

We have used chemical damage of the vein wall as a thrombogenic stimulus. This results in a thrombus which undergoes organization and shows no spontaneous thrombolysis or recanalization during a 5-week period of...
observation. Collateral pathways are opened up, the obstruction is adequately bypassed, and the animal shows no outward evidence of venous obstruction. Therapeutic defibrination (achieved with Arvin therapy) prevents thrombus formation in such an experimental model and allows the vein to be endothelialized again. In this respect it is more effective than heparin therapy, even when heparin is used in very large doses (10,000 units I.V. every 6 hours to 10 kg dogs). Lysis of preformed thrombus was not achieved with Arvin therapy even when the thrombus was less than 24 hours old.

As an experimental model the technic we have used offers many advantages. It is technically easy to perform. There is a high success rate for thrombus formation, the site and extent of which are reproducible. The thrombus is easy to study repeatedly and appears to have a histologic picture similar to that seen in man. The thrombus organizes and does not undergo spontaneous lysis or recanalization and thus allows anticoagulants and thrombolytic agents to be evaluated.

In the present experimental model the thrombus forms in a flowing stream of blood, there is endothelial damage, and the thrombus is adherent to the vessel wall. Damage to the vascular endothelium with exposure of collagen which serves as a focus for platelet-fibrin deposition is probably of considerable importance.12-13 The histologic picture of the thrombus in the present study is similar to that seen in man. However, one should still be cautious in applying all the results of the present study to clinical venous thrombosis for the following reasons: (1) The initial triggering event in the majority of patients is unknown. (2) The relative contribution of a variety of potential predisposing factors (such as blood stasis, altered blood coagulability, and damaged vascular endothelium) in patients is not known. (3) Assessment of the results of therapy of clinical venous thrombosis should take into account lysis of the thrombus and the prevention or reduction of thrombus propagation, pulmonary embolization, and the postphlebitic syndrome. Nevertheless, the present experimental model provides a convenient in vivo means of assessing anticoagulants and thrombolytic agents.

Therapeutic defibrination prevented the formation of a thrombus in these severely injured vessels and allowed the veins to become endothelialized. Presumably, because of the low amounts of circulating fibrinogen a stable platelet-fibrin mass could not form on the damaged endothelium and thrombus formation, at its earliest stage, did not occur. The unavailability of fibrin would also have ensured that if a thrombus had formed, even in the earliest stages it would not have been able to propagate. Heparin, even in massive doses (10,000 units I.V. every 6 hours to dogs weighing 10 kg) was not as effective as Arvin in preventing thrombus formation. Other workers have used heparin in dosages of 9,000 units/24 hours or less.14-16 The dosage of heparin we used should have been adequate, for even at the times of the lowest level of anticoagulation (immediately prior to a heparin dose being given) clotting times were prolonged to at least twice the normal level. Furthermore, 50% of the animals died of hemorrhage from the operative sites. Williams and Carey,17 by heparinizing the animal and applying an electric current to the external jugular vein as the thrombogenic stimulus, were able to prevent thrombus formation. These were acute experiments only, and there were no observations as to the subsequent development of thrombosis. Also, chemicals may result in a more severe form of venous injury than an electric current.

Increases in the activity of the blood-fibrinolytic system have been demonstrated with use of the venom of the Malayan pit viper,18 and there is some experimental evidence for the lysis of clot with its use.19 The crude venom used in the two studies just cited is not suitable for clinical use. The purified derivative Arvin causes changes in the blood-fibrinolytic system secondary to intravascular fibrin deposition; these changes are not likely to be of help in the lysis of preformed thrombi.6 Furthermore, thrombi are histologically different from clots,20 and the effects of
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_Circulation_. 1970;42:729-737
doi: 10.1161/01.CIR.42.4.729

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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