Intravenous Trypsin in Experimental Myocardial Infarction

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The possibility of using intravenous proteolytic agents in the treatment of acute myocardial infarction has been studied. The coronary arteries of closed-chest dogs were embolized with small repetitive doses of fibrin clots until definite electrocardiographic evidence of myocardial injury which persisted for at least two hours was observed. The treated animals were given up to six intravenous infusions of 250,000 Armour units of trypsin in 250 ml saline over a period of eight days, and the survivors were sacrificed on the ninth day. The control animals received no trypsin. These studies showed that trypsin caused dissolution of the host thrombus which formed around the fibrin clots, without damage to the infarcted tissue; that the coronary vessels were recanalized; that the extent of infarction was decreased; and that the electrocardiographic changes were improved and the mortality was reduced.

INTEREST in the use of intravenous proteolytic enzymes in the treatment of thrombotic diseases has gained new impetus with the advent of a crystalline preparation of trypsin for parenteral use. Innerfeld was the first to use intravenous trypsin for the treatment of human thrombotic diseases. However, to our knowledge, no animal experiments have been reported on the use of intravascular proteolytic agents for the treatment of arterial thrombi, particularly coronary arterial thrombi.

This paper is a report on results obtained from dogs treated with trypsin after experimental coronary artery embolization. The following questions have been investigated: (a) Can intravenous administration of a proteolytic enzyme (trypsin*) cause recanalization of the coronary vessels of the dog after embolization with fibrin particles; (b) is this type of treatment contraindicated in the presence of acutely infarcted myocardium because of the possibility of myocardial rupture; and (c) what blood pressure and electrocardiographic changes result from the administration of repeated large doses of trypsin under conditions of severe myocardial injury?

METHODS

Preparation of Fibrin Clots. 2000 N.I.H. units of bovine thrombin† were added to 250 cc. of reconstituted human plasma. The resulting clot was filtered and washed repeatedly with saline until reduced to a yellow-white fibrin network. This fibrin clot was then lyophilized, broken up into particles, and passed through a no. 40 U.S. Standard sieve. Only those particles of the fibrin clot were used which passed through this sieve, but not through a no. 50 sieve. These particles were then sterilized in an autoclave for 15 minutes at 20 pounds pressure.

Method of Coronary Embolization. Closed-chest dogs were embolized with fibrin particles by an adaptation of a previously reported technic.‡ By this technic emboli can be introduced directly into the coronary arteries of a closed-chest dog by means of a double-lumen steel catheter passed through the left carotid artery.

Experimental Procedure. The coronary arteries of 29 closed-chest dogs were embolized with repeated injections of fibrin particles (approximately 0.5 mg. per kilogram) until definite electrocardiographic evidence of myocardial injury was observed, as indicated by significant deviations of the RS-T

† Parke, Davis & Co., Detroit, Mich.
segments from control levels (table 3). The animals were then observed for a period of two hours to see if the electrocardiographic injury pattern persisted or progressed. Those animals in which the electrocardiogram tended to revert to normal were embolized further until persistent damage was seen. Animals which survived for less than 24 hours were not included in this series. Thirteen of the 29 animals injured in this manner were used indiscriminately as controls and returned to their cages; 16 animals were started on a course of treatment with intravenous trypsin. In the treated series 13 dogs each received from one to six intravenous trypsin infusions (250,000 Armour units in 250 cc. saline each) within a period of eight days. The remaining three dogs of this series were given much smaller amounts of trypsin and appear at the bottom of table 2. The rate of trypsin infusion was regulated in accordance with the blood pressure response of each animal and was adjusted to a rate which kept the blood pressure constant.

These rates varied from 10 to 60 drops per minute through a no. 24 needle. Strain gauge measurements of femoral arterial blood pressure, and 12-lead electrocardiograms of all animals were done daily during the course of the experiments. All dogs were given penicillin for the first three days after injury. Because of constant severe myocardial irritability (frequent ventricular extrasystoles or paroxysmal tachycardia), either intramuscular quinidine gluconate or intravenous Pronestyl was given to all dogs during this period. Surviving animals were sacrificed and autopsied. The presence or absence of visible clots in the epicardial coronary vessels was noted. These vessels with visible clots were perfused locally with saline as follows: A no. 26 needle was inserted into the lumen of the artery 1 to 2 cm. proximal to the site of the clot. Sulfur from a 2 cc. syringe was then perfused into the artery under a pressure of approximately 30 cm. Hg, and the passage of the perfusate was observed. In order to demonstrate clearly the pathway of the perfusate a solution made up of 6 per cent gelatine, 12 per cent sucrose and 0.25 per cent Evans' blue dye (T-1824) was used. This solution is dark blue, has a viscosity approximating that of blood, and does not extravasate from the vessels. Photographs were taken after perfusion of each vessel. After gross sectioning of the myocardium the extent of infarction was measured. This was classified as follows: mild, less

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**TABLE 2.**—Summary of 16 Dogs Treated with Intravenous Trypsin after Coronary Embolization with Fibrin Particles

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Amt. Trypsin Armour Units</th>
<th>Days Survived</th>
<th>Visible Clots</th>
<th>Vessels Patent</th>
<th>Degree of Infarction</th>
<th>% Change MAP Injury—8 Days</th>
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</thead>
<tbody>
<tr>
<td>255</td>
<td>4 × 250,000</td>
<td>8 yes</td>
<td>+</td>
<td>+</td>
<td>+8</td>
<td>+8</td>
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<tr>
<td>260</td>
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<td>++</td>
<td>++</td>
<td>+11</td>
<td>+11</td>
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<tr>
<td>261</td>
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<td>yes</td>
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<tr>
<td>267</td>
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<td>yes</td>
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<td>--20</td>
<td>--20</td>
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<tr>
<td>269</td>
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<td>+</td>
<td>+</td>
<td>--8</td>
<td>--8</td>
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<td>--15</td>
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<tr>
<td>314</td>
<td>6 × 200,000</td>
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<td>yes</td>
<td>+</td>
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<tr>
<td>338</td>
<td>5 × 200,000</td>
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<td>yes</td>
<td>++</td>
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<td>--1</td>
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<tr>
<td>340</td>
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<td>yes</td>
<td>++</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>284</td>
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<td>++</td>
<td>--11</td>
<td>--11</td>
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<tr>
<td>291</td>
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<td>yes</td>
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<td>--30</td>
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<tr>
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<td>yes</td>
<td>++</td>
<td>--7</td>
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</tr>
</tbody>
</table>

Average: --28.3%

* MAP = Mean arterial pressure.

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**TABLE 3.**—ECG Changes

<table>
<thead>
<tr>
<th>Initial Injury</th>
<th>Final ECG</th>
</tr>
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<tbody>
<tr>
<td>Severe</td>
<td>Moderate</td>
</tr>
<tr>
<td>Trypsin</td>
<td>5</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
</tr>
</tbody>
</table>

Note: Only animals surviving 9 to 10 days appear in this table.

**Mild Injury**—RS-T segment deviation of 1-2 mm.* **Moderate Injury**—RS-T segment deviation of 3-5 mm.* **Severe Injury**—RS-T segment deviation of over 5 mm.* * From control ECG, in more than one lead.

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* MAP = Mean arterial pressure.
than 15 per cent infarcted myocardium; moderate, 15 to 30 per cent; severe, 30 to 60 per cent; and massive, greater than 60 per cent. Hematoxylin and eosin stained serial sections were made of blocks taken from embolized vessels of both treated and control animals.

Two additional normal uninfarcted dogs were treated with massive infusions of trypsin over a period of eight days, one receiving 1,200,000 units in five infusions, the other receiving 500,000 units in four infusions. These dogs were sacrificed eight days after the start of the experiments and their vital organs (brain, heart, lung, liver, kidney, spleen and adrenal glands) were grossly and microscopically examined.

RESULTS

Embolization of the coronary arteries of the closed-chest dog with fibrin particles produced extensive myocardial infarction. These emboli did not resorb spontaneously within the selected eight-day postinjury period. Previous work on many animals embolized with plastic microspheres has shown that S-T segment shifts which persist for two hours or more were regularly associated with myocardial damage. Color cinematographs on three open-chest animals, and histologic sections on many animals, have proved that clots entered the coronary vessels and that extensive myocardial infarction resulted. Pale and cyanotic areas, "ballooning," and cardiac dilatation were present, as occurs after coronary ligation.

Sections were made of embolized vessels two hours after the introduction of fibrin particles. Serial sections of the coronary arteries of one dog of this series were examined. The findings were considered to be representative of the changes at the site of embolization of the entire group. The embolus of lyophilized fibrin appeared as an irregularly shaped, slightly eosinophilic, homogeneous body containing oval or round spaces of varying size, and scalloped, sharply defined margins. These spaces and scalloped margins were filled by a newly formed fibrin thrombus of the host in the two-hour control dog. This thrombus completely filled the residual lumen of the coronary artery and was propagated for a short distance proximally and distally.

On the day of injury, only two of the 29 experimentally embolized animals showed blood pressure falls which fulfilled the previously reported criteria for coronary shock. The average fall in mean arterial pressure for the entire series, two hours after the last injection of particles, was actually less than 5 per cent (range +15 to —30 per cent) of the preinjury level.

Control Series. Thirteen dogs were used as controls. Based on electrocardiographic evidence, these animals appeared to suffer initial myocardial injury comparable to, or milder than, the treated group (table 3). Eight of these control dogs showed severe to massive myocardial infarction at autopsy. Five dogs showed...
mild to moderate infarction. All control animals showed grossly visible clots in the epicardial arteries of both ventricles. The epicardial arteries were found to be occluded even when perfused under sufficient pressure to cause "ballooning" of the vessel and back flow around the needle puncture. In only one animal were the vessels found to be patent. Three dogs survived less than five days after embolization, and the remaining were sacrificed on the eighth to tenth postinjury day. These 10 dogs showed an average fall in mean arterial pressure of 27.4 per cent (range: −15 per cent to −50 per cent) from the level reached two hours after the last fibrin particle injection. These dogs manifested no other signs of shock and were active and ate well. The data are summarized in table 1.

Studies of sections of the nonperfusion vessels of control dogs showed them to be completely occluded by the fibrin emboli and the surrounding organizing host thrombus. Serial sections of embolized coronary arteries of one dog of this series were studied. The findings were considered to be representative of the changes at the site of embolization of this group. The fibrin emboli of the coronary arteries from the eight-day untreated dog exhibited fragmentation and lysis. Those fragments that persisted exhibited a lighter, faintly eosinophilic marginal zone and a slightly hematoxylin staining basophilism centrally. Marginal histiocytosis and multinucleated foreign-body giant cell formation were regularly observed. In some of the latter were found intracytoplasmic fragments of the emboli. The residual lumen of the artery, the scalloped margins and spaces within the emboli were filled by a dense, capillary-forming, endothelial proliferative process containing polymorphonuclear leukocytes. Endothelialization with the creation of some larger vascular channels were observed. However, serial sections ap-
FIG. 3. Photomicrographs of an epicardial coronary artery from an animal treated with six intravenous trypsin infusions of 200,000 Armour units each, after embolization with fibrin particles. (X 170.) A, fibrin particles, B, note absence of host thrombus.

FIG. 4. Electrocardiograms of a control dog embolized with fibrin particles. Initial—pre-embolization electrocardiogram. Injury—two hours after last injection of fibrin particles. Final—eight days postinjury.

Appear to indicate that this was yet a discontinuous, irregularly distributed process.

Treated Series. Sixteen dogs were treated with varying amounts of intravenous trypsin in saline. At autopsy 10 animals showed mild to moderate myocardial infarction and six showed severe to massive infarction. However, when the coronary vessels were perfused locally under a pressure of approximately 30 cm. H₂O, the perfusate was observed to flow past the
FIG. 5. Electrocardiograms of a dog treated with six intravenous infusions of 200,000 Units of trypsin each, after embolization with fibrin particles. Initial—preinjury control electrocardiogram. Injury—two hours after last injection of fibrin emboli. Final—the eighth postinjury day.

Serial sections of the site of embolization in one dog of this series were studied. The changes were considered representative of the findings for this group of animals. Microscopically, the emboli from the eight-day treated dog were essentially similar to those found in the untreated two-hour control animal, though the marginal scalloping seemed less distinct, thinned and "soft." This suggested that some marginal lysis had occurred. The most dramatic observation consisted in the disappearance of practically all evidence of the host thrombus. The only stigma of its having been present were the scattered, scanty foci of endothelial cells in some scalloped emboli-recesses in contact with the arterial intima. The residual lumen between the embolus and arterial wall appeared unaltered. The artery wall, except for the intimal changes observed above, showed no significant alterations.

The myocardial infarcts observed in the eight-day untreated dog were larger and fewer in number than those found in the treated dog. In the treated animal, they were smaller and more numerous, though the phase of organization by granulation tissue were similar in both animals. Calcification of the small infarcts was observed only in the treated dog. No inflammatory cellular reaction at the sites of infarction was observed in either animal.

None of the treated animals showed any adverse effects of the trypsin infusions as long as the rate of administration was adjusted so as to avoid a marked downward trend in blood pressure. There was a wide variation in this blood pressure response to trypsin. Some animals lost the hypotensive response after a short period of infusion, at 10 to 20 drops per minute, and the rate of infusion could then be safely raised to 50 to 60 drops per minute. Others (10 per cent to 15 per cent) remained quite sensitive to the hypotensive effects of trypsin and had to be carefully titrated at rates of 10 to 30 drops per minute during the entire course of the infusions. These variations were noted in the same animal on different occasions. There were no significant electrocardiographic changes due to trypsin infusion.
The two normal, uninfarcted dogs which received massive doses of trypsin showed no significant damage upon either gross or microscopic examination of all vital organs including the brain.

DISCUSSION

The closed-chest technic for embolizing the coronary arteries of dogs with fibrin clots offers a means of investigating the effects of intravenous proteolytic agents in experimental acute coronary occlusion. The differences in the results between the control and treated group of dogs were consistent. This is particularly significant since the electrocardiographic evidence suggests that the treated group appeared to receive more severe initial myocardial injury than did the control group. All of the observations support the impression that the dogs of the treated group were benefited by the trypsin infusions.

The observed perfusability of the embolized arteries of treated animals as compared to the nonperfusability of the untreated group was considered of paramount importance and was therefore explored in several ways. An attempt was made to demonstrate differences in the filling of the vascular trees by standard injection technics like the lead-agar method, radio-opaque vinylite, and Dock's solution. The latter was by far the most technically satisfactory, but in most instances the vessels of untreated control animals filled so well that roentgenograms gave no indication of the marked damage to the circulation. Because of the well-known extensive collateral circulation in the dog heart, it was not even possible to demonstrate consistent differences in coronary filling between normal and injured dog hearts.

In order to obviate the problems presented by the extensive collateral circulation and the attendant retrograde flow observed during total coronary perfusion, local perfusion of embolized arteries was used as the criterion of physiologic patency. The Dock's solution was modified by the addition of Evan's blue dye so that color photographs could be taken of the perfused vessel.

The coronary arteries of the treated group were all patent. This finding has been substantiated by histologic studies which suggest that the recanalization of these coronary arteries occurs by digestion of the host thrombus which formed in and around the fibrin particles. The extent of infarction was decreased; the electrocardiographic changes were improved; and the blood pressure, on the eighth postembolization day, had essentially returned to normal. The two animals which were in shock showed impressive responses to intravenous trypsin as shown by complete recovery of the animal in all respects. On the other hand, in the control series, the emboli were found to occlude the coronary vessels in all but one case; the extent of infarction was greater; the electrocardiographic changes showed no essential improvement; and the blood pressure, on the eighth postembolization day, was an average of 27.4 per cent lower than the reference level recorded two hours after the last injection of fibrin emboli.

The question of whether dissolution of the host thrombus is due directly to the proteolytic activity of the infused trypsin or is manifested through the fibrinolytic or other systems is yet to be investigated.

As pointed out by Guest and co-workers and others, different species show marked dissimilarities in their fibrinolytic systems and in their response to intravenous trypsin. This fact probably accounts for the lack of adverse effects found in our experiments as compared with the marked toxic changes in the rabbit as recently reported by Taylor and colleagues. For this same reason, the results obtained in our dog experiments cannot yet be applied to humans. On the other hand, clinical reports to date on approximately 1,500 patients have shown none of the severe toxic effects found in the rabbit. It is felt that our experiments demonstrate that a proteolytic agent can significantly aid the dissolution of coronary arterial thrombi without further damage to the infarcted myocardium, thus greatly strengthening the hope that an effective agent may be found for human use.

CONCLUSIONS

Intravenous administration of a proteolytic enzyme (trypsin) in closed-chest dogs caused
the recanalization of coronary arteries embolized with fibrin particles. There was little or no effect upon the fibrin particles themselves. Complete occlusion of vessels embolized in this manner was found to occur in two hours or less due to the formation of a host thrombus in and around the fibrin particle. Trypsin appeared to act upon, or initiate action upon, this secondary thrombus so as to cause its removal. Thus, treated animals had physiologically patent coronary arteries while the arteries of untreated animals remained occluded.

Such treatment is apparently not contraindicated in dogs in the presence of severe myocardial infarction; in no instance has myocardial rupture occurred. These dogs were active during the postinfarction treatment period. No toxic or adverse effects, except hypotension, were observed during the infusions; hypotension could be avoided by careful control of the infusion rate. Microscopic studies of the infarcts showed no interference with the healing process.

Electrocardiographic abnormalities produced by infarction were often completely reversed following trypsin infusions, and drops in blood pressure were significantly less in the treated group after an eight-day period.

REFERENCES
