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Prevention of Postoperative Adhesions in the Dog by Intravenous Injections of Plasminogen Activators.* (21921)

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Recent studies have indicated that experimental venous thrombi in the rabbit(1), and arterial thrombi in the dog(2) can be successfully liquefied *in vivo* by the intravenous administration of plasminogen activators.‡ Activation of animal plasminogen was carried out in the above studies by large amounts of streptokinase (SK). In addition, in the dog, plasminogen activation was carried out by injections of a mixture of small amounts of SK and a partially purified human plasminogen preparation(2). This latter method was based on the observation that the activation of animal plasminogen by SK was markedly facilitated by the presence of small amounts of human plasminogen preparations(5).§

The purpose of this report is to present evidence that, in dogs with an experimental traumatic peritonitis, intravenous injection of large amounts of streptokinase alone, or of a mixture of small amounts of SK and a human plasminogen preparation was capable of preventing or modifying the production of postoperative abdominal adhesions.

Materials and methods. Sixty-three mongrel dogs weighing from 10 to 15 kg were used in these experiments. A traumatic hemorrhagic fibrinous peritonitis was produced by scarifying a 2 x 2 inch area of peritoneal wall with a vegetable grater according to the technic of Wright and associates(6). Immediately following surgery, each animal was injected intramuscularly with 1,000,000 units of a long acting penicillin (Bicillin®). The animals were divided into 3 groups: 23 controls, 32 animals injected intravenously with SK‖ alone; and 8 animals injected intravenously with a mixture containing 25,000 units of SK and 60 mg of a partially purified human plasminogen preparation.¶ Of the animals receiv-

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‡ Plasminogen refers to the naturally occurring precursor of plasmin, the proteolytic enzyme of mammalian plasma(3). Other terminology, in current use, includes profibrinolysin for the precursor and fibrinolysin for the active enzyme(4).

§ While 200 units of SK per ml of dog plasma was required to activate significant amounts of plasminogen, the addition of minute amounts of human plasminogen preparations to dog plasma reduced the SK requirement to approximately 20 units per ml of dog plasma(2).

‖ A commercial preparation (Varidase) Lot No. 7-1089-413, containing streptokinase and streptodornase, and obtained through the courtesy of Dr. J. M. Ruegsegger of Lederle Laboratories. This preparation also contains streptococcal hyaluronidase as well as other contaminating substances.

¶ A lyophilized human plasminogen preparation prepared from human plasma Fraction III (Cohn) by the Christensen and Smith technic(7). The preparation contained 4% nitrogen and had a proteolytic assay, following streptokinase activation, of 4.2 casein units per mg nitrogen(8).

TABLE I. Summary of Results of I.V. Plasminogen Activators on Prevention of Postoperative Adhesions in Dogs.

Animals	No. of dogs	Results*† No. with dense adhesions	No. with moderate adhesions	No. with no adhesions
Controls	23	19	4	0
SK alone—total group	32	10	5	17
400,000 u. od x 6	2	1	1	0
300,000 u. bid x 2	7	4	0	3
300,000 u. bid x 3	12	5	2	5
400,000 u. bid x 3	11	0	2	9
SK + human plasminogen prep.‡	8	0	6	2

* Classified according to thickness, firmness of adherence, and extent of adhesions as follows: Dense—thick, firmly adherent and covering entire area of abrasion. Moderate—thin, more loosely adherent, and scattered. None—occasional filmy to no adhesions.

† Dividing the entire data into 2 classes (*i.e.*, adhesions vs. no adhesions) and employing the chi square test (R. x 2 table; using Yates' correction) statistical analysis showed a highly significant difference between all treatment groups together as compared to the control animals (P less than .01).

Because it was believed that a trend was present relating SK dosage to suppression of adhesion formation, chi square testing of the controls against the combined 3 lower daily dose levels and against the highest daily dose level was carried out. Results indicated that the first 3 treatment groups combined, differed from the control in a manner to which little significance could be ascribed ($P = 0.05$). The last treatment group however showed a highly significant difference from the control (P less than .001).

The authors are indebted to Dr. Stanley Lang for the statistical treatment of the data.

‡ Injection mixture contained 25,000 u. SK and 60 mg of partially purified human plasminogen preparation.

ing SK alone, 2 were given a single injection of 400,000 units of SK daily for 6 days; 7 received 300,000 units twice daily for 2 days; 12 were injected with 300,000 units twice daily for 3 days; and 11 were given 400,000 units twice daily for 3 days. The animals receiving the activation mixture of SK plus the human plasminogen preparation were given single daily injections for 3 days. Treatment with the plasminogen activators was begun on the first postoperative day. Venous specimens of whole blood were obtained just prior to and 10 minutes following each injection of the plasminogen activator. The specimens were incubated at 37°C in a water bath and observed for clotting and subsequent lysis of the clot. The animals were sacrificed 12 days postoperatively and the extent and nature of the adhesions were noted. Specimens of the traumatized site, heart, lungs, kidneys, liver and spleen were taken for histological study.

Results. A summary of the results is shown in Table I. All of the 23 control animals developed adhesions, dense in 19, and moderate in 4. In the group of 32 animals receiving SK alone, 17, or approximately half, developed no adhesions, 5 had moderate, and 10 had dense adhesions. The prevention of adhesions was related to the daily dose of SK rather than the duration of treatment, with the best results occurring with the highest dose level of SK. Nine of 11 animals, receiving 400,000 units twice daily for 3 days, did not develop any adhesions. In the group of 8 animals receiving the mixture of 60 mg of the partially purified human plasminogen preparation and 25,000 units of SK, 2 did not develop adhesions, and the other 6 developed moderate adhesions. None of the animals of this latter group exhibited dense adhesions.

In order to define more clearly the effect of I.V. streptokinase on the peritoneal exudate, 3 control animals, and 3 animals receiving 400,000 units SK bid for 3 days, were sacrificed on the fifth postoperative day and the abraded sites examined histologically. Each of the control animals showed an extensive fibrinous exudate with evidence of rapidly developing organization. The exudate in each of the SK-treated animals was minimal with little evidence of organization. The striking

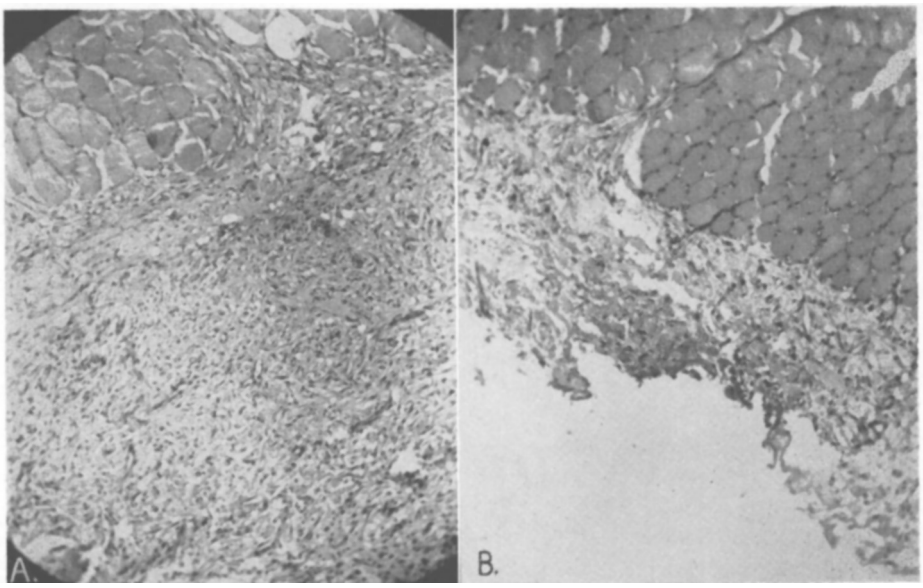

FIG. 1. Effect of I.V. SK on experimental traumatic peritonitis in the dog (see text). Specimens taken from abraded area on 5th postoperative day. A. Control animal. Note thick organizing granulation tissue adjacent to striated muscle fibers in cross section with penetration between muscle bundles (magnification 67 ×). B. SK treated animal. Thin layer of granulation tissue with little evidence of organization (same magnification).

histological difference in a control as compared to an SK-treated animal is shown in Fig. 1.

Each of the blood specimens removed prior to the injection of the activators clotted and did not significantly lyse over an observation period of 48 hours. Following the injection of SK alone, the blood clotted normally and subsequently lysed completely in from one to 4 hours.** Following injection of the mixture of SK and the human plasminogen preparation, the blood either did not clot, or the clot was poorly formed and rapidly lysed over the next 30 minutes. In several instances, determinations were made of plasma fibrinogen, plasminogen, and circulating proteolytic activity, with results similar to those previously reported with these 2 methods of plasminogen activation(2).

A finding of interest observed in the animals receiving SK alone related to the laparotomy wound. Preliminary studies revealed a high incidence of wound dehiscence in animals receiving large doses of SK alone (300,000 units bid). The dehiscence was attributed to an accelerated resorption of the catgut sutures. With the use of silk sutures, as utilized in the studies reported, no wound dehiscence was noted. However, in the animals treated with the largest daily dose of SK alone (400,000 units bid), fresh hemorrhage into the incisional wound was observed during the 3 days of SK therapy. Following the cessation of SK administration, the hemorrhage rapidly resorbed. At the time of final exploration, the wounds were well healed and did not differ from the control wounds.

No other significant toxic manifestations were noted in any of the animals. All the animals survived the total observation period. Studies of the sections of heart, lung, kidney and liver were not remarkable.†† In the majority of the animals, sections of the spleen revealed passive congestion but the presence of congestion was independent of treatment.

Discussion. The prevention of postopera-

** Lysis times of 1-2 hours were observed after the injection of 400,000 units of SK and 3-4 hours after the injection of 300,000 units of SK.

†† We are indebted to Dr. Philip Wasserman, Department of Clinical Laboratories, Jewish Hospital of Cincinnati, for these studies.

tive abdominal adhesions in many of the animals receiving the intravenous injection of plasminogen activators probably indicates a lysis of the traumatic inflammatory exudate. It is highly unlikely that the effects of the plasminogen activators can be entirely attributed to a lack of formation of a fibrinous exudate since therapy was not instituted for a full day following trauma. The presence of increased capillary permeability in the traumatic area may account for the appearance of plasmin or of SK at the site of exudation. Although the streptokinase preparation contained streptodornase and streptococcal hyaluronidase, both of which may contribute to the lysis and resorption of the exudate, we have stressed the fibrinolytic system since fibrin is the major insoluble component of the experimental exudate in this study.

The differences observed with the 2 types of activators used in this study, and the special fibrinolytic activity seen after *in vivo* injections of SK alone, have been previously noted (2). Recent studies indicate that the mixture of SK plus the human plasminogen preparation supplies the activator for the animal plasminogen(5,9). The special fibrinolytic effects seen with large doses of SK alone are related to the adsorption of SK on fibrin, and the ability of SK to suppress antiplasmin activity(10). The resultant of these latter phenomena is to provide an "inhibitor free" fibrin for the action of activated plasmin(10).

Conclusion. In dogs with an experimental traumatic peritonitis, the intravenous injection of streptokinase alone in large doses, or of a mixture of smaller amounts of SK plus a partially purified human plasminogen preparation, prevented or modified the development of postoperative adhesions.

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Electrophoretic Distribution of Hexosamine in Plasma Proteins of the Rat Following Thyroidectomy.* (21922)

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Plasma hexosamine levels increase in the rat following either thyroidectomy or hypophysectomy(1). The cause appears to be a deficiency of thyroid hormone in both instances. Hexosamine is present in all plasma protein fractions but is richest in the globulins(2). Since globulins have been reported to be elevated in rats following thyroidectomy (3), electrophoretic studies were carried out to establish which protein fractions were responsible for the plasma hexosamine changes observed in the rat following thyroidectomy.

Methods. Male Sprague-Dawley rats, weighing 55-65 g, were individually caged, fed a high calcium diet(4), and permitted to drink water *ad libitum*. Thyroidectomy was per-

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