Heparin in the Treatment of Experimental Peritonitis

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Two experiments were performed to determine the effect of heparin on experimental fibrinopurulent peritonitis in dogs. Peritonitis was induced by the creation of a 10 cm long isolated loop of terminal ileum. In a first experiment comprising 24 dogs the necrotic loop was removed 24 hours later without cleaning or irrigating the peritoneal cavity. All dogs showed fibrino-purulent peritonitis at that time. No antibiotics were given. All dogs received 500 ml of Ringer's lactate during surgery and were allowed p.o. fluids on the first postoperative day. At the time of excision the dogs were blindly randomized into a control group and two treatment groups receiving heparin 100 u/kg i.p. or s.c. respectively. Of the eight animals in the control group, five died of peritonitis and two showed residual intraperitoneal sepsis at the time of sacrifice 14 days after the initial surgery. Thus, only one dog cleared his peritoneal infection spontaneously. Of the heparin treated dogs six out of eight in the i.p. treated and seven out of eight in the s.c. treated group cleared their peritonitis spontaneously within 14 days ($p \leq 0.05$ and 0.02 respectively). In a second experiment peritonitis was induced in 24 dogs as described above, but the necrotic loop was not removed. The dogs were blindly randomized to daily low dose heparin (50 u/kg s.c. b.i.d.) or no therapy. Only two out of 12 dogs of the control group survived the observation period of 14 days compared with eight out of 12 of the heparin treated group ($p \leq 0.05$). However, in all dogs in this experiment residual i.p. sepsis was found. We conclude that heparin has a therapeutic effect in experimental canine peritonitis by preventing the additional apposition of fibrin and, thus, rendering the bacteria more susceptible to cellular and noncellular clearing mechanisms.

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DESPITE ANTIBIOTICS, blood transfusions and modern anesthesia, peritonitis is still a serious disease especially in patients whose antibacterial and immunological defenses have been compromised (*i.e.* the aged, patient with cancer, uremia, hepatic insufficiency, nephrotic syndrome and patients receiving immunosuppressive drugs). The source of peritoneal contamination, its quantity, the organisms involved and the prior condition of the peritoneal cavity are all factors which help to determine the severity of infection, its localization, its spontaneous resolution and its response to treatment. Nevertheless, the mortality of large bowel perforation in almost all series exceeds 20%, in patients above 70 years the mortality is even above 50%.[6,11,20,29]

It seems desirable to develop additional modalities in the treatment of peritonitis beyond surgery and antibiotic treatment. A number of lines of evidence favor the use of anticoagulation as an adjunct for treatment of peritonitis. Zinsser and Pryde[30] have shown that bacteria are more rapidly cleared from the peritoneal cavity in heparinized animals, and survival in acute experimental peritonitis has been shown to depend on the rapid clearing of bacteria.[8] Despite the failure of Kay and Lockwood to alleviate peritonitis with small doses of heparin in 1947,[16] we attempted to influence the outcome of experimental peritonitis in dogs by anticoagulation with heparin.

Materials and Methods

Experiment 1

Twenty-four mongrel dogs of either sex weighing 15–20 kg were used. The dogs were anesthetized with tiamynal and succinylcholine and after sterile preparation and draping the abdomen was entered through a midline incision. Peritonitis was induced according to the method described by Rosato.[26] A segment of 10 cm of terminal ileum ending 10 cm proximal to the ileo–cecal valve was freed up, but the mesenteric attachments were left intact. The continuity of the bowel was then re-established by a one-layer end-to-end anastomosis of the two ends of ileum using 4-0 silk and the defect in the mesentery was then closed with interrupted 4-0 silk sutures. The ends of the isolated loop were closed in one layer using 4-0 silk and finally the blood supply to the isolated loop was ligated (Fig. 1). During the operative procedure the dogs received 500 ml of Ringer's lactate but no antibiotics were given either intra- or postoperatively. After 24 hours the dogs were re-operated and on exploration showed signs of severe, fibrinopurulent peritonitis originating from the ischemic loop and ex-

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tending over the entire lower and in some cases upper abdomen (Fig. 2). The cultures of the peritoneal exudate revealed uniformly *B. fragilis, E. coli,* Streptococcus D and in some cases diphteroid bacteria and Clostridia. During this operation the necrotic loop of ileum was removed and the abdomen was closed without any debridement or irrigation of the peritoneal cavity. Again the dogs received 500 ml of Ringer's lactate during surgery, but no antibiotics. Before the second operation the dogs were blindly randomized into a control group and two treatment groups. The two treatment groups received a single injection of heparin, 100 units/kg either intraperitoneally or subcutaneously. Dogs not receiving heparin got an equal volume of normal saline subcutaneously and/or intraperitoneally. After surgery all dogs were allowed p.o. fluids *ad libitum* the first postoperative day and had free access to food starting the third postoperative day. All animals dying of any cause were autopsied, surviving animals were sacrificed 14 days after surgery and autopsy was performed with special reference to remaining intraperitoneal infection.

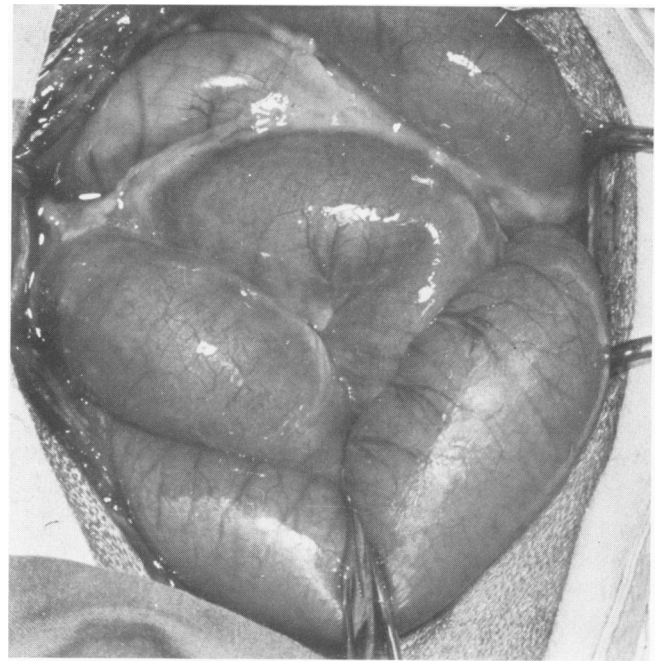

FIG. 2. Severe fibrinopurulent peritonitis has developed 24 hours after the creation of the ischemic loop of ileum.

Experiment 2

In this experiment 24 mongrel dogs of either sex weighing 15–20 kg were used. Peritonitis was induced as described above. The dogs received again 500 ml of Ringer's lactate during the operation but no antibiotics. However the second operation to resect the necrotic loop of terminal ileum was not carried out and the ischemic loop was left in place. The day after surgery the dogs were randomized into two groups, a control group and a group receiving heparin 50 u/kg b.i.d. subcutaneously. The postoperative treatment and the autopsies performed were identical to those of the dogs in Experiment 1. All dogs surviving 14 days were killed at that time and autopsied.

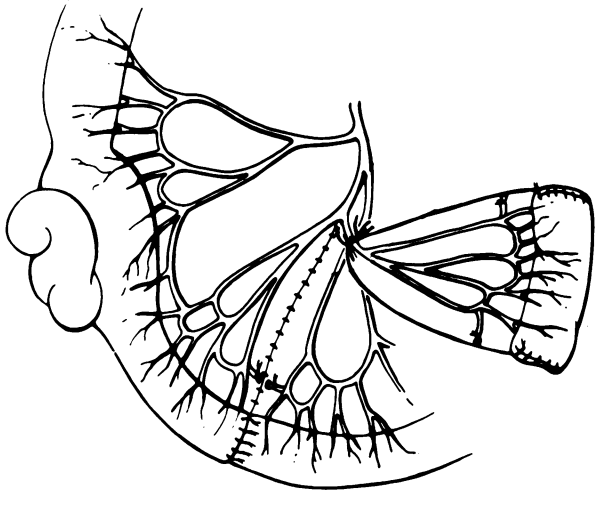

FIG. 1. The model of peritonitis utilized in these experiments was originally reported by Rosato (1974). A 10 cm segment of terminal ileum, 10 cm proximal to the ileocecal valve has been freed up and the blood supply has been ligated. The ischemic loop is left *in situ*. The continuity of the gastrointestinal tract has been reestablished with a one-layer end-to-end anastomosis.

Results

Experiment 1: The Effect of Heparin on Peritonitis in the Absence of Necrotic Bowel

The results of this experiment are recorded in Table 1. Of the eight dogs in the control group five died of peritonitis within five days after surgery. At autopsy they all showed severe fibrinopurulent peritonitis extending over the entire abdomen. Two dogs survived the observation period but still showed evidence of intraperitoneal infection at the time of sacrifice. The intraperitoneal infection presented as intermesenteric abscess in both cases. Only one dog of the control group survived the observation period and resolved its peritonitis spontaneously. Six of the eight dogs treated with intraperitoneal heparin and seven of the eight dogs treated with subcutaneous heparin survived the full 14 day observation period. None of these 13 dogs showed any evidence of remaining peritonitis or intraperitoneal abscesses at the time of sacrifice 14 days after surgery. There were only minimal adhesions and the peritoneum was clear and

TABLE 1. *Effect of Heparin (100 u/kg) on Survival of Dogs with Fibrinopurulent Peritonitis*

Group	# of Dogs	Died of peritonitis	Survived with peritoneal infection	Survived without i.p. infection
Control	8	5	2	1
Heparin i.p.	8	2	—	6*
Heparin s.c.	8	1	—	7†

* $p \leq 0.05$ compared with the control group.
† $p \leq 0.02$ compared with the control group.

transparent. Two dogs in the group treated with intraperitoneal heparin and one dog of the group treated with subcutaneous heparin died of peritonitis during the observation period and showed essentially the same peritoneal findings as the dogs in the control group. The intestinal anastomoses of all dogs in the control as well as the treatment group did not show any evidence of disruption and were well healed at the time of sacrifice. The statistical analysis of the results of the Chi-square test revealed that the survival in both treatment groups were statistically significantly better ($p \leq 0.05$ and $p \leq 0.02$ respectively) when compared with the control group.

Experiment 2: The Effect of Heparin on Peritonitis in the Presence of Necrotic Bowel

The survival curve of the dogs treated with continuous subcutaneous heparin is shown in Figure 3. Statistical analysis by the chi-squared test revealed that the survival in the treated group was significantly better than in the control group one and two weeks after surgery ($p \leq 0.05$). Eight out of 12 dogs treated with heparin survived the 14 day observation period, whereas only two out of 12 dogs in the control group survived. The dogs dying during the observation period again showed severe fibrinopurulent peritonitis originating from the ileal loop in the lower abdomen and extending over the entire abdominal cavity. In contrast to the dogs in Experiment 1, whose necrotic bowel had been resected, all surviving dogs in this experiment showed residual intraperitoneal sepsis mostly in the form of intramesenteric abscess walled off by mesentery, bowel loops and omentum. The abscess was usually small in surviving animals treated with heparin and rather large in animals belonging to the control group. The bacteriologic findings were the same as described in Experiment I (*B. fragilis, E. coli*, Streptococcus D). Outside of the abscess the abdominal cavity of treated surviving dogs was free of peritoneal sepsis. The intestinal anastomoses in all treated and untreated dogs were intact.

Discussion

Clinical peritonitis is frequently characterized by a fibrinous or fibrinopurulent exudate. Fibrin formation is an essential early step in wound healing and the fibrinous exudate which is a common response to all peritoneal trauma and inflammation is usually seen as the first step to the sealing of perforations of various hollow organs. Once sealed, adhesions form and permanent closure can sometimes be spontaneously achieved. Surgically induced wounds of the intestinal tract, for example, are classically closed by serosa–serosal approximation so that fibrin sealing will take place and a water tight closure may be obtained. Fibrin, thus, has long been considered a peritoneal defense.

At the present state of our knowledge the following series of events leads to formation of fibrin and subsequently to fibrinous and fibrous adhesions. If the peritoneal serosa is injured, peritoneal mast cells respond with the release of histamine and other "permeability factors" such as nucleosides and polypeptides.[27] These substances cause an increase in vascular permeability mainly in the small venules and a protein rich, fibrinogen containing plasma is exuded into the peritoneal cavity.[19] Since the injured cells also release thromboplastin fibrinogen is converted into fibrin. The normal peritoneum has an inherent fibrinolytic

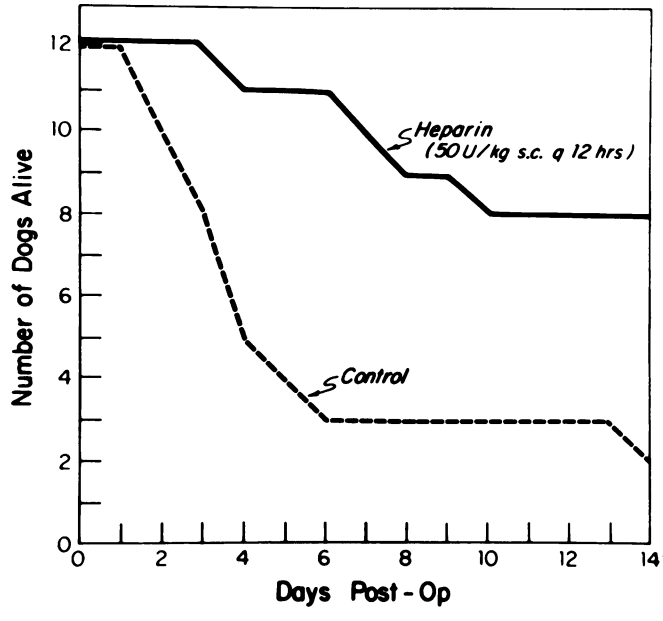

FIG. 3. Survival curve of dogs in which the necrotic loop of bowel has not been removed. Dogs treated with heparin (50 units/kg subcutaneously every 12 hours) solid line, control group interrupted line. The survival of the heparin treated group is significantly better after one and two weeks ($p \leq 0.05$).

activity as demonstrated by Benzer.[2] This fibrinolytic activity is based on the plasminogen activator present in the mesothelial and submesothelial cells[21,23] and fibrinous adhesions are probably normally removed from the peritoneal cavity by fibrinolysis and absorption.[12,22] However, injury to the peritoneum also causes a depression of its fibrinolytic activity.[7] Buckman[3] has recently shown that abrasion or crushing causes a depression of the peritoneal plasminogen activator activity by 30%. Such depression may permit the persistence of fibrinous adhesions until fibroblasts can lay down collagen leading to fibrous adhesions. We have recently shown that peritonitis nearly totally abrogates peritoneal fibrinolytic activity so that fibrin probably is slow to be resorbed after peritonitis.[9]

Though fibrin is apparently responsible for the sealing of wounds, the question can be raised whether it is helpful or harmful once the wounds have been sealed by the surgeon. Does it interfere or assist at the elimination of organisms, processes which involved nonphagocytic absorption from the peritoneal cavity as well as chemotaxis, phagocytosis and intracellular killing of the bacteria by polymorphonuclear leukocytes and monocytes? One might expect that the smooth circulation of fluid in the peritoneal cavity responsible for sweeping the cavity and clearing organisms through the diaphragmatic lymphatics might be inhibited, that fibrin might trap and isolate bacteria, and that phagocytosis might be adversely affected. Some clinical evidence that fibrinous exudate may be detrimental to recovery from peritonitis has recently been offered by Hudspeth.[10]

Some of the information which led to these experiments derives from experiments on adhesion formation. The role of anticoagulation in preventing the formation of fibrinous adhesions has long been under study.[15,28] The first successful attempt to interfere with the formation of fibrin and thus prevent peritoneal adhesion formation in experimental animals was reported by Lehman.[18] It was found that intra-abdominal adhesions caused by mechanical damage, and peritoneal contamination could be prevented by intraperitoneal administration of heparin in the dog and the rabbit. More recently, Knightly[17] has reported that heparin alone, and in combination with fibrinolysin, was effective in the prevention of adhesions after peritoneal irritation in the rat. Benzer[2] was able to prevent peritoneal adhesion formation after talcum insult by intraperitoneal administration of 12,000 units of streptokinase. James[14] did similar experiments in rats and rabbits and found that large doses and prolonged periods of treatment were required. Bryant[4] showed that the injection of 300,000 units of fibrinolysin at the time of creation of 20 circular defects of the wall of the jejunum reduced the severity and frequency of adhesion formation significantly. Buckman[5] showed that ancrod, a proteolytic enzyme derived from pit viper venom which causes total defibrinogenation in the experimental animal, was able to completely prevent adhesion formation after creation of an avascular peritoneal patch.

There are, however, only a few reports in the literature where the effect of anticoagulants and fibrinolytic enzymes on bacterial peritonitis and the clearance of bacteria from the peritoneal cavity have been evaluated. The earliest report by Kay and coworkers[16] who administered 200 units of heparin systemically 12 hours after the induction of peritonitis in dogs did not show any beneficial effect on the outcome. Zinsser and Pryde[30] mentioned, however, that bacteria are cleared faster from the peritoneal cavity in heparinized animals. In our experiments we were able to show that a one time dose of 100 units of heparin per kg given either intraperitoneally or subcutaneously is able to increase the survival in experimental peritonitis in dogs significantly. In addition the administration of heparin prevents the formation of intraperitoneal abscesses and adhesions when the source of peritoneal infection, in our case ischemic segment of ileum, is removed. If the source of infection is not removed heparin again improves the survival rate of experimental peritonitis in dogs significantly, but is not able to prevent abscess formation.

The mechanism of action of heparin in experimental peritonitis is not clear. The most likely explanation is that heparin acts as an anticoagulant, preventing the deposition of fibrin and the entrapment of bacteria within this fibrin, thus, rendering the bacterial organisms more susceptible to both nonphagocytic absorption from the peritoneal cavity and phagocytic destruction. One also should keep in mind, however, that the systemic rather than the local intraperitoneal effects of heparin might be beneficial in the outcome of animals suffering from experimental peritonitis. For example, subclinical disseminated intravascular coagulation may accompany severe peritonitis and septic shock—heparin may antagonize this effect though we did not notice any clotting abnormalities in our dogs and the doses of heparin given to the animals were below the therapeutic level and comparable to low dose heparinization in the clinical situation. Heparin is released from the mast cells of dogs in anaphylactic shock in amounts sufficient to cause prolongation of the clotting time.[13] However,

it has been pointed out that, for several reasons, the dog is unique in its ability to release heparin so easily.[25] Heparin may accelerate bacterial clearance by preventing the thrombosis of the subperitoneal lymphatics. Heparin is also known to interact with the complement system, by enhancing the C1-esterase inhibition resulting in consumption of the early complement components and activation of C4 and C2.[24] It has also been shown to have a beneficial effect on acute renal failure caused by intraperitoneal sepsis.[1]

Whatever the mechanism, both large single and repeated small doses of heparin appear to benefit the animal with severe intraperitoneal sepsis. If these results are confirmed in other models by other investigators heparin may be worthy of trial as an adjunct in clinical peritonitis.

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Addendum

Since submission of this paper O'Leary et al. have confirmed the beneficial effects of heparin on experimental peritonitis in the rat (*Surg. Forum* 28:55, 1977).