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Noxythiolin (Noxyflex), aprotinin (Trasylol) and peritoneal adhesion formation: an experimental study in the rat

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SUMMARY

Clinical and experimental studies have suggested that noxythiolin and aprotinin may prevent intraperitoneal adhesion formation. A comparison was therefore made of their efficacy in preventing the reformation of adhesions following surgical lysis in a controlled trial using rats. Neither noxythiolon nor aprotinin had any significant benefit over surgical lysis alone. The mortality rate was high in the noxythiolin-treated group.

INTRAPERITONEAL adhesions are a common cause of intestinal obstruction (Ellis, 1974). Numerous methods have been tried over the years in an attempt to prevent adhesions, but with little success, despite initial enthusiastic reports. Recently, two further substances have been reported as efficacious in decreasing the incidence of intraperitoneal adhesions, namely noxythiolin (Noxyflex, Geistlich) and aprotinin (Trasylol, Bayer). Following the observation of Tolhurst Cleaver et al. (1974) that adhesions occurred less frequently in rats given intraperitoneal lavage with noxythiolin than those given lavage with Hartmann's solution, Gilmore and Reid (1976) showed that a single intraperitoneal dose of noxythiolin reduced the incidence of adhesions in rats. Experimental studies (Vorster, 1968; Grundmann, 1969) have shown that aprotinin reduces the incidence of adhesions and Mooney (1976) has shown a reduced incidence of adhesions in a clinical study. However, these studies all relate to prevention of adhesions at an initial laparotomy. As Ellis (1971) has pointed out, the vast majority of adhesions are harmless and may well be protective or even life-saving to the patient, for example in protecting an anastomotic line in which the blood supply was endangered or in localizing infection. In the study reported here no attempt was made to prevent adhesions forming following the initial laparotomy, but, following a second laparotomy to divide the adhesions created at the initial laparotomy, either noxythiolin or aprotinin was instilled into the peritoneal cavity in order to assess the efficacy of these substances at preventing the reformation of adhesions.

Materials and methods

Adult male Sprague–Dawley rats weighing 250–300 g were used in this investigation. Anaesthesia was induced with ether and maintained with a nitrous oxide, halothane and oxygen mixture. At the initial laparotomy the abdomen was opened through a midline incision 5 cm long. Adhesions were created by a method of multiple peritoneal trauma. The operative techniques were as follows.

A defect 2 cm × 0·5 cm was made in the left flank, involving removal of the peritoneum and underlying layer of muscle within this area. The defect was repaired with a continuous 3/0 chromic catgut suture. A similar defect was made on the right flank, but this was not sutured. The caecum was delivered from the wound and its surface rubbed briskly with sterile gauze until petechial haemorrhages were observed. The peritoneum at the ileocaecal junction was grasped between forceps and stripped off the antimesenteric border of the ileum for about 5 cm from the ileocaecal junction (Glucksman, 1966), removing a strip of peritoneum about 1 mm wide and also the underlying layer of longitudinal muscle. The abdominal incision was closed in two layers, catgut sutures being placed through the musculoperitoneal layers and silk sutures through the skin.

The rats were subjected to a second laparotomy 2 weeks later. The position of all adhesions was noted and the adhesions divided with scissors. At the end of this procedure, just prior to closure of the abdomen, 2·5 ml of one of a batch of numbered solutions (1–100) was instilled into the peritoneal cavity. Surviving animals were killed 2 weeks later and inspected for adhesions. The position of all adhesions was noted. At completion of the experiment the number code of the solutions was broken. In total, 120 animals were operated on and 100 received an intraperitoneal solution at the completion of the second laparotomy. The following solutions were instilled into the peritoneal cavity: 20 rats received 2·5 ml of normal saline, 20 received 2·5 ml of 2·5 per cent Noxyflex (noxythiolin + amethocaine), 20 received 2·5 ml of 2·5 per cent Noxyflex S (noxythiolin alone), 20 received 25 000 units of aprotinin and 20 received 50 000 units of aprotinin. Statistical analysis of the results was carried out using Fisher's exact test.

Results

Some adhesions formed in each animal following the initial laparotomy. As each group of animals acted as their own 'controls' in assessing the results of the various treatments, statistical analysis was carried out to assess whether there was any significant difference between the number of adhesions in each control group prior to treatment. None was found.

The sites of adhesion formation and the number of animals having adhesions at the various sites are shown in *Tables I–VI*. Following the second laparotomy there was a significantly increased mortality in the group treated with Noxyflex ($P<0·001$) and also in the group treated with Noxyflex S ($P<0·005$). None of the treatment groups showed a significant reduction in adhesions compared with surgical lysis alone. On the contrary, there was a significant increase in adhesions to the left flank in the group treated with Noxyflex S ($P<0·005$). It was not possible to evaluate statistically the effect of Noxyflex on adhesions due to the high mortality in this group. Post-mortem examination of the 2 rats that died in the saline-treated group showed that death occurred from peritonitis resulting from caecal volvulus and gangrene of the caecum. All the rats that died in the groups treated with Noxyflex and Noxyflex S did so within 48 h of operation. Limited post-mortem examination revealed no abnormality in the peritoneal cavity, but showed bilateral pleural effusions and haemorrhagic lesions in the lungs.

Discussion

The results show that none of the substances used in an attempt to prevent adhesions has a clear advantage

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over surgical lysis alone. Ideally, any substance tested should totally prevent adhesions. Reduction in the number of adhesions or their length of attachment is probably not significant since it is apparent from clinical experience that a single band adhesion can be just as troublesome in terms of intestinal obstruction and strangulation as numerous smaller adhesions.

The experimental model was deliberately designed so that no attempt was made to prevent adhesions at the initial laparotomy. As Ellis (1971) has stated, adhesions are vascular grafts to areas of doubtful viability and it is uncertain whether we should aim to prevent adhesions after a first laparotomy. Where we should aim to prevent adhesions is in the patient undergoing a second or subsequent laparotomy for intestinal obstruction due to adhesions where the bowel is viable and where, after dividing the adhesions, we seek to prevent them reforming.

Sutured peritoneal defects have been shown to act as an adhesive stimulus (Ellis, 1962; Gilmore and Reid, 1976), but in this study adhesions formed to the defect in only about one-third of animals compared with all animals as reported by Gilmore and Reid (1976). This may relate to the fact that the defect was sutured with catgut rather than silk as in the study of Gilmore and Reid (1976). It should, however, be noted that more adhesions formed to the sutured defect in the left flank than to the unsutured defect in the right flank following the initial laparotomy, thus confirming the work of Ellis (1962) who showed that sutured peritoneal defects acted as a stimulus to adhesion formation. Also, in contrast to Gilmore and Reid's study (1976), no reduction in adhesion formation was found with noxythiolin and also the mortality rate was high.

Noxythiolin is manufactured in two forms: Noxyflex which contains 10 mg of amethocaine in each 2·5 g of noxythiolin and Noxyflex S which contains no amethocaine. After breaking the code it was found that all except one animal in the Noxyflex group died and also 8 of those in the Noxyflex S group. Tolhurst Cleaver et al. (1974) reported 100 per cent mortality in a group of rats with faecal peritonitis treated with Noxyflex. No satisfactory explanation could be found for this at the time, but recently it has become apparent that the amethocaine contained in Noxyflex may be toxic to rats. The convulsant dose given intravenously to rats is 1·5 mg/kg body weight (Edwards, 1978). The volume of solution given to the rats, i.e. 2·5 ml, contains 0·25 mg, giving a dose of approximately 1 mg/kg given intraperitoneally. Further ether may raise the toxicity of amethocaine up to tenfold and ether was used to induce anaesthesia and therefore could have taken the dose of amethocaine past the convulsant dose to the lethal dose. Although this provides a satisfactory explanation for those animals treated with Noxyflex, it does not explain the 40 per cent mortality in the animals treated with Noxyflex S which does not contain amethocaine. It must therefore be assumed that noxythiolin in the concentration used is absorbed rapidly through the large areas of peritoneal defect and is toxic to rats. The discrepancy between these results and those of Gilmore and Reid (1976) who had no mortality may be due to the fact that they used a sutured wound only to stimulate adhesions and there were no large raw areas of peritoneum such as those

Table I: SURGICAL LYSIS ALONE

Site of adhesion	No. animals with adhesions: Initial laparotomy ($n = 20$)	After lysis ($n = 20$)
Right flank	1	1
Left flank	10	0
Caecum	20	14
Ileum	18	15

Deaths = 0; % survivors having adhesions = 90.

Table II: SURGICAL LYSIS PLUS SALINE

Site of adhesion	No. animals with adhesions: Initial laparotomy ($n = 20$)	After lysis + saline ($n = 18$)
Right flank	0	0
Left flank	6	2
Caecum	18	16
Ileum	17	16

Deaths = 2; % survivors having adhesions = 100.

Table III: SURGICAL LYSIS PLUS NOXYFLEX (2·5 PER CENT)

Site of adhesion	No. animals with adhesions: Initial laparotomy ($n = 20$)	After lysis + Noxyflex ($n = 1$)
Right flank	0	0
Left flank	6	0
Caecum	17	1
Ileum	16	1

Deaths = 19; % survivors having adhesions = 100.

Table IV: SURGICAL LYSIS PLUS NOXYFLEX S (2·5 PER CENT)

Site of adhesion	No. animals with adhesions: Initial laparotomy ($n = 20$)	After lysis + Noxyflex S ($n = 12$)
Right flank	2	2
Left flank	10	6
Caecum	15	12
Ileum	16	12

Deaths = 8; % survivors having adhesions = 100.

Table V: SURGICAL LYSIS PLUS TRASYLOL (25 000 UNITS)

Site of adhesion	No. animals with adhesions: Initial laparotomy ($n = 20$)	After lysis + Trasylol ($n = 20$)
Right flank	1	0
Left flank	7	1
Caecum	19	15
Ileum	17	16

Deaths = 0; % survivors having adhesions = 85.

Table VI: SURGICAL LYSIS PLUS TRASYLOL (50 000 UNITS)

Site of adhesion	No. animals with adhesions: Initial laparotomy ($n = 20$)	After lysis + Trasylol ($n = 20$)
Right flank	0	0
Left flank	6	4
Caecum	20	18
Ileum	20	18

Deaths = 0; % survivors having adhesions = 100.

in the present study through which noxythiolin may be rapidly absorbed. Also Gilmore and Reid (1976) used only 1 per cent noxythiolin, compared with 2·5 per cent in the present study.

Animals treated with aprotinin showed no reduction in adhesion formation compared with surgical lysis alone. This does not support the work of Vorster (1968) and Grundmann (1969) who showed that adhesions produced by peritoneal abrasion and instillation of talc could be reduced by a single intraperitoneal injection of aprotinin. However, it should be noted that in these studies adhesions were not completely abolished (Vorster, 1968), but their area of attachment was reduced (Grundmann, 1969). Certainly there are good theoretical reasons for believing that aprotinin should reduce adhesions since it has been shown to reduce the inflammatory response in peritoneal wounds created by cautery and to 'prevent the development of inflammatory granulation tissue' (Grundmann, 1969). Two recent clinical papers have also suggested that aprotinin reduces adhesions. Mooney (1976) treated 20 patients with intraperitoneal aprotinin and at a 'second look' procedure, i.e. laparotomy or laparoscopy, there was a 'marked reduction' in adhesions in 16 of the patients. However, this was an uncontrolled trial. More recently, Perovic et al. (1978) have compared a single dose of intraperitoneal aprotinin with no such treatment in perforated appendicitis in children. The presence of adhesions was indirectly assessed by comparing the incidence of 'mechanical ileus' in the two groups. It was significantly higher in the untreated group. However, the absence of 'mechanical ileus' does not necessarily prove absence of intraperitoneal adhesions.

The results obtained in the experimental model certainly do not support the claims that a single intraperitoneal dose of either noxythiolin or aprotinin prevents adhesions.

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