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Experimental adhesion prophylaxis with recombinant tissue plasminogen activator

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The deposition of fibrin in the peritoneal cavity leads to fibrous adhesion formation. Recombinant tissue plasminogen activator (rtPA), delivered locally, was investigated as a method of preventing adhesion formation. Six standardised areas of peritoneal ischaemia were formed in each of 36 male Wistar rats randomised to three intraperitoneal treatments: (A) no treatment control; (B) carboxymethylcellulose gel; (C) rtPA–carboxymethylcellulose gel combination. At 1 week all animals underwent relaparotomy and the number of ischaemic sites with an adhesion counted by an independent observer.

rtPA-treated animals formed fewer adhesions compared with gel alone or controls (median number of adhesions 1.5 *versus* 2.5 *versus* 5, $P < 0.001$, ANOVA). Intraperitoneal rtPA in a slow-release formulation is able to reduce adhesion formation significantly in an animal model and may prove to have clinical benefit.

Intra-abdominal adhesions are the most common cause of small bowel obstruction in developed countries with an appreciable morbidity and mortality *(1)*. Adhesions develop as a result of the organisation of the fibrin-rich inflammatory exudate released after peritoneal injury *(2)*. It has previously been demonstrated that both animal and human peritoneum possesses fibrinolytic activity *(3,4)* and suggested that this plays a central role in preventing adhesion formation by lysing fibrinous deposits *(5)*. Peritoneal fibrinolytic activity is reduced by mechanical trauma, ischaemia and bacterial and chemical injury—all conditions associated with adhesion formation *(6–9)*. The principal mediator of fibrinolytic activity in human peritoneum has been identified as tissue plasminogen activator (tPA) *(10)*. After peritoneal inflammation, plasminogen activator inhibitor-one (PAI-1), not found in normal peritoneum, is detected and this results in loss of peritoneal fibrinolytic activity.

Recombinant tPA (rtPA) could thus be used as a competitive antagonist of PAI-1 to enhance peritoneal fibrinolytic activity in conditions that lead to adhesion formation. This may then promote lysis of the fibrin deposits that occur after peritoneal injury and reduce adhesion formation.

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Materials and methods

rtPA preparation

Twelve aliquots (5 ml) of aqueous recombinant tissue plasminogen activator (rtPA, Boehringer Ingelheim, Bracknell, UK) were prepared at a concentration of 1 mg/ml. These aliquots were used to reconstitute 1.5% methylcellulose powder (Sigma Chemical Co., Poole, UK) to produce rtPA gels of viscosity 2250 cps buffered to pH 7.4. To act as controls 12×5 ml aliquots of methylcellulose gel alone at the same viscosity were also prepared. The 24 aliquots were drawn up into individual syringes, numbered randomly and stored at 4°C.

Animal model

Ischaemic peritoneal buttons were formed in 36 adult male Wistar rats (250–300 g). Under ether/air anaesthesia

the abdomen was opened in the midline. A button of parietal peritoneum adjacent to the paracolic gutter was picked up in fine forceps and the base ligated with 3/0 chromic catgut (Ethicon® Ltd, Edinburgh, UK). Three ischaemic buttons were created along each paracolic gutter. At the completion of the laparotomy each animal was randomised to one of three groups: (A) no treatment control; (B) gel alone; (C) rtPA–gel. Randomisation was performed by a third party with the operator unaware of each group. The appropriate numbered syringe, the contents of which were unknown to the operator, was then instilled intraperitoneally in animals in groups (B) and (C). The abdomen was closed in two layers; continuous 3/0 PDS (Ethicon Ltd, Edinburgh, UK) to the muscle layer and continuous 3/0 Nurolon® (Ethicon Ltd, Edinburgh, UK) to the skin. The skin was then sprayed with povidone iodine. Animals were returned to their cages, recovered and allowed food and water *ad libitum*.

After 1 week all animals underwent a second laparotomy. Adhesions were scored by the operator unaware of the treatment group to which each animal belonged: the code being held by the assistant. Adhesions were scored as being present or absent to an ischaemic button, giving an objective adhesion score of 0–6 for each animal.

Statistical method

Kruskal–Wallis one-way analysis of variance and the Mann–Whitney *U* test was used for comparison between groups.

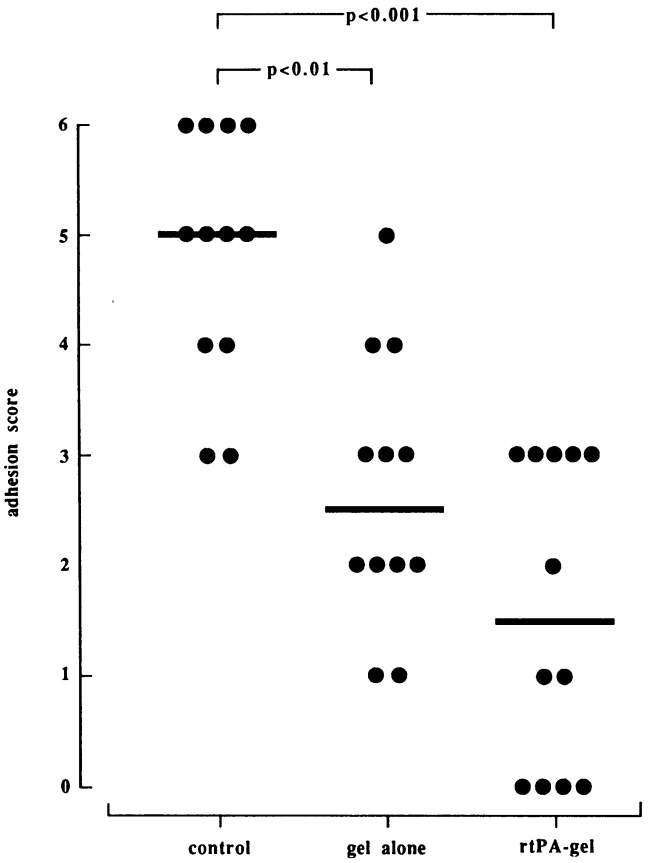

Figure 1. Adhesion scores for control, gel alone and rtPA–gel groups (bars indicate median score).

Results

The adhesion score for each animal is shown in Fig. 1. For the no treatment control animals the median number of adhesions per animal was 5 (range 3–6), for the gel alone treated group 2.5 (range 1–5) and for the rtPA–gel treated animals 1.5 (range 0–3). These differences were statistically significant for the treatment arms compared with the control (control *vs* gel alone, $P < 0.01$; control *vs* rtPA–gel, $P < 0.001$). There was no statistically significant difference between the gel alone and the rtPA–gel groups, but a clear trend to further reduction in adhesion score with the rtPA–gel group.

No adverse effect was noted in any animal; in particular, laparotomy wounds were soundly healed and there was no evidence of haemorrhage. At the second laparotomy there was neither residual evidence of the gel nor any excess free peritoneal fluid.

Discussion

Although intraperitoneal rtPA–gel reduced adhesion formation significantly in this model it is of interest that the inert carrier, methylcellulose, also reduced adhesions compared with no treatment control animals. Sodium carboxymethylcellulose has been used in other studies and shown to reduce intra-abdominal adhesions in the rat *(11–13)*. As this agent is physiologically inert it was concluded that carboxymethylcellulose probably acted mechanically as both a barrier and lubricant. Further studies of macromolecules, including dextran and carboxymethylcellulose, have suggested a mode of action in which methylcellulose produces a polymeric coating on the peritoneal surface which increases local activity of intrinsic plasminogen activator *(14)*.

Fibrinolytic agents, other than tPA, have also been investigated previously. Using a peritoneal abrasion model in dogs or rabbits, streptokinase solutions have been shown to reduce the incidence of adhesions *(15–17)*. However, with an ischaemic button model in rats, no reduction in adhesion formation was found when streptokinase was given for up to 72 h *(18)*. Streptokinase-activated plasminogen has been found to reduce adhesion formation when given as a single intraperitoneal dose *(19,20)*, but these results were not corroborated by other studies *(21,22)*. More recently, urokinase has been found to be ineffective in adhesion prevention when given either intraperitoneally or intravenously *(23)*. The failure of these agents may be related to their relative lack of specificity for fibrin compared with tPA and that in aqueous solution they are rapidly absorbed across the peritoneal cavity.

In rabbit and dog models rtPA has been shown to reduce both primary adhesions after surgery or reforma-

tion of adhesions after initial division *(24–26)*. In a study in rabbits, no alteration in plasma fibrinogen or tPA levels was detected in blood samples taken at 2 h *(27)*. Delivery of rtPA by a continuous infusion pump *(28,29)* or intermittent intraperitoneal injection *(30)* has also proved effective and demonstrated no additional benefit beyond 4 days. This time period coincides with the recovery of intrinsic peritoneal plasminogen-activating activity after a single peritoneal insult *(31)*.

Using a similar model to the one we describe, Evans *et al. (32)* showed a reduction in adhesion formation in rats, but also a 50% reduction in wound hydroxyproline content, which might indicate a deleterious effect on wound healing. However, more sophisticated tensiometric measurements of wound healing and anastomotic bursting pressure showed no adverse effect of intraperitoneal rtPA at 7–10 days *(33)*.

Intraperitoneal rtPA has also been used successfully in a rat model to prevent intra-abdominal abscess formation after bacterial peritonitis *(34–36)*. Similarly, fibrin accumulation after intraocular surgery may be prevented by local delivery of exogenous rtPA *(37,38)*.

These studies all indicate that rtPA delivered locally to body cavities can limit fibrin formation where this may be detrimental. In the present study of intra-abdominal adhesions, rtPA in a slow-release gel was shown to significantly reduce adhesion formation. The viscous gel alone led to reduction in adhesions and a further reduction occurred with the incorporation of rtPA. It is likely that two mechanisms are at work: the methylcellulose acts as a lubricating barrier, possibly enhances local mesothelial tPA, and rtPA lyses any fibrinous adhesions that form.

The use of rtPA in a slow-release gel as a method of adhesion prevention or prophylaxis is very attractive. Because the action of tPA is localised to fibrin deposits, fibrinolytic activity is limited to this site, which prevents indiscriminate fibrinolysis. Further studies of wound healing and serial measurement of systemic fibrinolytic levels are required to determine fully if there are any adverse effects of this potential new treatment.

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