Dextran and adhesions in guinea-pigs

M. J. ten Kate-Booij, H. J. van Geldorp and A. C. Drogendijk

Department of Obstetrics and Gynaecology, Erasmus University, 3015 GD Rotterdam, The Netherlands

Summary. This prospective, randomized, 'blind' study with guinea-pigs was performed to assess the possible benefit of 6% dextran 70 (molecular weight 70 000) in the prevention of post-operative intra-abdominal adhesions and recurrent adhesions after adhesiolysis. In 50 guinea-pigs lesions for inducing adhesions were applied at the end of the uterine horn. On the right side a strip lesion was made and on the left side an end-to-end anastomosis was performed after section. Before closing the peritoneum 20 ml 6% dextran 70 (N = 25) or saline (N = 25) were introduced into the peritoneal cavity. A second laparotomy 4 weeks later showed no differences in adhesion formation in the animals treated with 6% dextran 70 and saline. In the animals with adhesions adhesiolysis was performed and 6% dextran 70 or saline was left in the peritoneal cavity. Again no beneficial effect of dextran was seen. The end-to-end procedure appeared to be far more suitable for producing adhesions than was the strip lesion.

Introduction

Every infertility surgeon tries to prevent adhesions as much as possible, because adhesions localized around the ovaries and Fallopian tubes disturb the ovum pick-up mechanism. Healing of the peritoneum occurs as long as the vascularization remains intact (Ellis, 1971; Ryan, Groberty & Majno, 1971). Injuries within the peritoneal cavity produce fibrinogen and a fibrin clot is formed. The fibrin is absorbed and peritoneal repair occurs by differentiation of new mesothelium from the underlying connective tissue, which is probably mediated by fibroblasts. Infection, drying of the serosa, contact with blood (a second source of fibrin; Ryan *et al.*, 1971), denudation of the surface and foreign bodies disturb the normal repair mechanism. These pathological circumstances accompanied by vascular damage leading to ischaemic tissue may inhibit fibrinolysis and adhesions are formed (Ellis, 1971). The chance of adhesion formation is diminished when microsurgical techniques, involving gentle tissue handling, meticulous haemostasis, working with special micro-instruments and non-reactive suture material, are used. Continuous lavage during the operation prevents tissue drying and blood clotting. The contribution of adjuvant medication, intraperitoneally or systemically, to adhesion prevention is still not clear. Many agents have been studied (Holtz, 1980). Theoretically their effect is based on reducing the inflammatory reaction or the formation of fibrin. Dextran solutions appeared to be the most promising and have become very popular in infertility surgery, although such use is still controversial.

Previous studies with dextran (Neuwirth & Khalaf, 1975; Luengo & van Hall, 1978; Utian, Goldfarb & Starks, 1979; di Zerega & Hodgen, 1980; Holtz, Baker & Tsai, 1980; Soules, Dennis, Bosarge & Moore, 1982; Vemer, Boeckx & Brosens, 1982) are not easily compared, because of the many variables, including differences in experimental methods, types of lesion and in dosage and, often, study groups that are too small. Only a few studies have been reported that investigated adhesion reformation after lysis (Kapur, Gulati & Talwar, 1972; Seitz, Schenker, Epstein & Garcia, 1973; Holtz & Baker, 1980), although this mimics the clinical situation. It is not always clear whether the observations have been done 'blind' and there is no uniformity in the manner of adhesion score and evaluation. Only recently have microsurgical techniques been employed in the studies. Because of the uncertainties with the above studies we have investigated the effect of

dextran on adhesion formation and on adhesion reformation after lysis. We also investigated the efficacy of the type of lesion for inducing adhesion formation.

Materials and Methods

Fifty (50) female adult non-pregnant guinea-pigs with an average weight of 800 g were used. They were anaesthetized with an i.m. injection of ketamine-HCl (Ketalar: Parke, Davis), 50 mg/ml, and 2% xylazine (Rompun: Bayer, Brussels, Belgium) (0.2 ml/kg body weight) and the abdomen was opened along the midline. Each female received preoperatively i.m. 0.3 ml 25% chloramphenicol (250 mg/ml: Intervet, Boxmeer, The Netherlands).

Before starting all visible talc was removed from the surgical gloves with saline solution (0.9% (w/v) NaCl). A standardized lesion was made at the distal end of both uterine horns near the junction with the oviducts. On the right anterior side a raw surface was produced by removing about 20 × 4 mm of peritoneum, called a 'strip lesion'. No attempt was made to prevent the minimal bleeding. On the left side the uterine horn was microsurgically transected with subsequent end-to-end anastomosis with monofilament nylon sutures, 8-0 (Dermalon, Davis & Geck, Hants, U.K.). The sutures were situated at such a distance from each other that minimal peritoneal defects remained. Avoidance of prolonged drying of the tissue during the operation was achieved by continuous lavage with saline (0.9% (w/v) NaCl). Before closing the peritoneum, 20 ml of the experimental dextran solution (6 g dextran with an average molecular weight of 70 000 in 100 ml saline: Macrodex: Pharmacia, Uppsala, Sweden) were introduced into the peritoneal cavity. The treatments with dextran or saline were allocated at random (25 animals/group). The abdominal wall was closed in two layers. The second laparotomy was performed 4 weeks later. Microscopic observation and prescription of the adhesions present were carried out by the same person (H. J. van G.), who was unaware of the initial treatment. In the guinea-pigs with adhesions, adhesiolysis was performed microsurgically and they were again allocated to a dextran or saline group. After 4 weeks another operation was performed and the adhesions present were scored in the same manner. Some of the animals without adhesions on the anterior strip lesion side received a new strip lesion, but now localized at the medial side of the uterine horn bordering the mesosalpinx fat.

The scheme of the experiment is shown in Text-fig. 1. The degree of adhesion was scored with a modified Hulka score (Hulka, Omran & Berger, 1978) and included the nature and the extent of adhesions, i.e. Grade 0, no adhesions; Grade 1, filmy, mostly avascular adhesions, easily separable and limited to one area; Grade 2, thick, vascularized adhesions, leaving a raw surface after division, limited to one area; and Grade 3, numerous extensive adhesions. Each uterine horn was graded separately. If there was a combination of lesions of Grades 1 and 2, the higher grading value was used.

For statistical analysis of the data, the Yates-Cochran test and the Sign test were employed.

Results

Two of the 50 animals died soon after the first operation (wound infection), leaving 48 animals for the first examination. Two animals in the end-to-end dextran group were excluded because of local infection (Text-fig. 1). The results shown in Text-fig. 1 indicate that treatment with the dextran solution was not more effective than the saline for either type of lesion. The scores for adhesions on the left and on the right sides were frequently different for the same animal. Only a small number of animals had the same adhesion score for both sides (2, 3 and 3 for Grades 0, 1 and 2 respectively with dextran treatment and 0, 1 and 1 for saline treatment). Many more adhesions were seen with the end-to-end anastomosis than with the strip lesion (Text-fig. 1): 33 of the 48 strip lesions induced

Text-fig. 1. Scheme of the experiment and the results

Adhesions§

Examination II

*Died soon after the laparotomy.
†2 excluded because of local infection.
‡1 excluded because of defective apparatus.
¶1 died because of a leg infection.
§Values in parentheses indicate nos. with Grade 1 and Grade 2 lesions respectively.

Table 1. Experimental studies with dextran 70 on adhesion formation

Authors	Animal	Lesion	Dosage (ml/kg)			No. of animals		Microsurgery and lavage controls	Blind study	Adhesiolysis performed	Beneficial
			6%	32%		Control	Treated				
Neuwirth & Khalaf (1975)	Rabbit	Denudation, transecting	—	20		8	13	No	?	No	Yes
Luengo & van Hall (1978)	Pig	Transecting, wedge ovary	—	+ spongostan		20	20	No	?	No	Yes
di Zerega & Hodgen (1980)	Monkey	Scraping, crushing	—	2		5	5	No?	?	No	Yes
Holtz <i>et al.</i> (1980)	Rabbit	Abrasion, crushing	—	2-5		5	5	No	Yes	No	Yes
Holtz & Baker (1980)			—	5-10		5	5	No	Yes	Yes	No/yes (high dosage)
Utian <i>et al.</i> (1979)	Rabbit	End-to-end anastomosis	—	10		10	10	Yes	?	No	Yes
Soules <i>et al.</i> (1982)	Rabbit	Cutting, scraping	10	—		10	10	Yes	?	No	Yes
Vemer <i>et al.</i> (1982)	Rabbit	Neostomy, end-to-end anastomosis	—	5		5	5	Yes	Yes	No	No
Present study	Guinea-pig	Strip-lesion, end-to-end anastomosis	20	—		25	25	Yes	Yes	Yes	No

no adhesions. Of these 33 animals, 19 were given a new medial-sided strip lesion and 8 developed adhesions.

Adhesiolysis procedures were performed on 58 uterine horns: 2 animals died because of wound infection, both in the saline group, and 1 animal in the dextran group had a progressive infection of the leg and died. The apparatus failed for another dextran group animal. There was again no beneficial effect from the use of dextran (Text-fig. 1).

Discussion

Dextran is a polysaccharide with a high viscosity. When dextran solutions are applied intraperitoneally, their mode of action (Polishuk & Aboulafia, 1967) could be due to: (1) mechanical separation of the organs; (2) coating raw surfaces; and (3) altering the structure of fibrin, which makes it more susceptible to lysis (Tangen, Wik, Almquist, Arfors & Hint, 1972). Possibly there is also the effect of dilution of clotting and other tissue factors promoting adhesion formation. The available solutions are different in concentration and molecular weight: 10% dextran of M_r 40 000, 6% dextran of M_r 70 000 and 32% dextran of M_r 70 000. Dextran 40 seems without benefit because of the rapid absorption from the peritoneal cavity. Table 1 details the studies with dextran 70, mostly using rabbits. The lesions were applied to the uterine horn or oviduct and included transecting with or without anastomosis or cutting, stripping, scraping, denudation and drying of the surface. All kinds of lesion induced adhesion formation. In our study the strip lesion was less suitable for inducing adhesions. This could have been because enough vascularization remained for peritoneal repair (Ellis, 1971). Transecting the uterine horn with subsequent anastomosis is a good method for inducing adhesions in the guinea-pig and the rabbit. Soules *et al.* (1982) observed more adhesion formation with the cut lesion than with the scrape lesion, but di Zerega & Hodgen (1980) saw no difference between the scrape and crush lesion. Holtz *et al.* (1980) noticed more dense adhesions in the combination of crushing with abrasion, than with abrasion alone. Therefore, both the type of lesion and the sort of animal seem to be important for adhesion formation. Our own observations gave us the impression that the localization of the lesion to fatty tissue perhaps influenced the scar tissue formation. For this reason 19 animals received a medial sided strip lesion bordering the mesosalpinx fat and 8 developed adhesions. However, no firm conclusions can be drawn, because there was no control group for this treatment.

Several authors (see Table 1) have reported a reduction of adhesion formation in animals treated with 32% dextran 70. Utian *et al.* (1979) found no difference in adhesion formation (and fertility rates) between the 6% and 32% groups. Holtz *et al.* (1980) reported a reduction of adhesions with a low dose of 32% dextran after a peritoneal injury, but there was no reduction of adhesions after lysis and treatment with the low dose. A larger dose was necessary to inhibit adhesions after lysis (Holtz & Baker, 1980). Of the studies associated with a beneficial effect of dextran, only that by Utian *et al.* (1979) used a lavage control, and this could have influenced the results. With a high dose of 6% dextran 70 (20 ml/kg body weight) we saw no difference in adhesion reformation after lysis between the treated and the control group. We used a higher dose and a lower concentration than did Holtz & Baker (1980) and Holtz *et al.* (1980) in their studies. Holtz *et al.* (1980) and Luengo *et al.* (1978) suggested that a low dose is not able to produce a purely mechanical effect and that a larger dose may extend the duration of action. The earlier observations about the beneficial effect of dextran 70 could not be confirmed, for 6% or for 32% concentrations, by Soules *et al.* (1982), Vemer *et al.* (1982) and the present study. Conflicting data still exist about the use of dextran solutions in the prevention of adhesions in women. In the clinical situation, adhesions already exist, mostly after an infection which may have damaged the endosalpinx. There have been only a few clinical studies with high molecular weight dextran solutions (Adhesion Study Group, 1983; Rosenberg & Board, 1984) and the evaluation is often difficult because of the variables in the clinical material and the necessity of a second examination. When 32% dextran was

used in women undergoing infertility operations, a reduction in the formation of adhesions overall and in the reformation after lysis was reported (Rosenberg & Board, 1984), but more extensive clinical studies are desirable.

This study was supported by a grant from the foundation 'Dr Saal van Swanenbergstichting', Leiden, The Netherlands.

References

- Adhesion Study Group** (1983) Reduction of postoperative pelvic adhesions with intraperitoneal 32% dextran 70: a prospective, randomized clinical trial. *Fert. Steril.* **40**, 612–619.
- di Zerega, G.S. & Hodgen, G.D.** (1980) Prevention of postoperative tubal adhesions: comparative study of commonly used agents. *Am. J. Obstet. Gynec.* **136**, 173–178.
- Ellis, H.** (1971) The cause and prevention of postoperative intraperitoneal adhesions. *Surg. Gynec. Obstet.* **133**, 497–511.
- Holtz, G.** (1980) Prevention of postoperative adhesions. *J. Reprod. Med.* **24**, 141–145.
- Holtz, G. & Baker, E.** (1980) Inhibition of peritoneal adhesion-reformation after lysis with thirty-two percent dextran 70. *Fert. Steril.* **34**, 394–395.
- Holtz, G., Baker, E. & Tsai, C.** (1980) Effect of thirty-two percent dextran 70 on peritoneal adhesion formation and reformation after lysis. *Fert. Steril.* **33**, 660–662.
- Hulka, J.F., Omran, K. & Berger, G.S.** (1978) Classification of adnexal adhesions: a proposal and evaluation of its prognostic value. *Fert. Steril.* **30**, 661–665.
- Kapur, B.M.L., Gulati, S.M. & Talwar, J.R.** (1972) Prevention of re-formation of peritoneal adhesions. *Arch. Surg.* **105**, 761–764.
- Luengo, J. & van Hall, E.V.** (1978) Prevention of peritoneal adhesions by the combined use of spongostan and 32% dextran 70: an experimental study in pigs. *Fert. Steril.* **29**, 447–450.
- Neuwirth, R.S. & Khalaf, S.M.** (1975) Effect of thirty-two percent dextran 70 on peritoneal adhesion formation. *Amn. J. Obstet. Gynec.* **121**, 420–422.
- Polishuk, W.Z. & Aboulafia, Y.** (1967) Dextran in prevention of peritoneal adhesions. *Israel J. Med. Sci.* **3**, 806–808.
- Rosenberg, S.M. & Board, J.A.** (1984) High-molecular weight dextran in human infertility surgery. *Am. J. Obstet. Gynec.* **148**, 380–384.
- Ryan, G.B., Groberty, J. & Majno, G.** (1971) Postoperative peritoneal adhesions. *Am. J. Path.* **65**, 117–140.
- Seitz, H.M., Schenker, J.G., Epstein, S. & Garcia, C.R.** (1973) Postoperative intraperitoneal adhesions: a double blind assessment in their prevention in the monkey. *Fert. Steril.* **24**, 935–940.
- Soules, M.R., Dennis, L., Bosarge, A. & Moore, D.E.** (1982) The prevention of postoperative pelvic adhesions: An animal study comparing barrier methods with dextran 70. *Am. J. Obstet. Gynec.* **143**, 829–834.
- Tangen, O., Wik, K.O., Almquist, I.A.M., Arfors, K.E. & Hint, H.C.** (1972) Effects of dextran on the structure and the plasmin-induced lysis of human fibrin. *Thromb. Res.* **1**, 487–492.
- Utian, W.H., Goldfarb, J.M. & Starks, G.C.** (1979) Role of dextran 70 in microtubal surgery. *Fert. Steril.* **31**, 79–82.
- Vemer, M., Boeckx, W. & Brosens, I.** (1982) Use of dextrans for the prevention of postoperative peritubal adhesions in rabbits. *Br. J. Obstet. Gynaec.* **89**, 473–475.

Received 31 December 1984