

## A comparative study of the effects of thrombin and electrodesiccation used for haemostasis on inflammation and adhesion formation

H.Yarali<sup>1,4</sup>, V.Gomel<sup>2</sup>, D.Uygur<sup>1</sup>, C.Öncüoğlu<sup>1</sup>, G.Gediköglü<sup>3</sup> and T.Gürkan<sup>1</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Faculty of Medicine, Hacettepe University, Sıhhiye, Ankara, Turkey 06100, <sup>2</sup>Department of Obstetrics and Gynecology, Faculty of Medicine, University of British Columbia, Vancouver, Canada and <sup>3</sup>Department of Pathology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

<sup>4</sup>To whom correspondence should be addressed

**Electrodesiccation or chemical agents, such as thrombin and fibrin sealant, may be used to control oozing in the peritoneal cavity. Electrodesiccation is time consuming and associated with adjacent thermal damage. Adhesion formation remains a concern with the use of thrombin and fibrin sealant. In this study, adhesion formation and various histological parameters of inflammation were evaluated following haemostasis with electromicrodesiccation or thrombin in the rabbit model ( $n = 36$ ). Following laparotomy, the right uterine horn was subjected to a measured injury producing sufficient oozing. After the injury was effected, the animals were randomized to haemostasis with electromicrodesiccation ( $n = 18$ ) or thrombin ( $n = 18$ ). In the first phase of the study, the histological parameters of acute injury and haemostasis with either modality were evaluated in two animals in each group. In the second phase, one, two and 10 animals, in each group, were submitted to second-look laparotomy on post-operative days 2, 7, and 15, respectively and the type and extent of adhesions were quantified. Histological parameters of inflammation as well as the type and extent of adhesions were comparable between the two groups. We conclude that local application of thrombin is not associated with a statistically greater degree of post-operative adhesions when compared to electromicrodesiccation.**

**Key words:** adhesion/electrodesiccation/haemostasis/inflammation/thrombin

### Introduction

The approaches available to control diffuse areas of capillary bleeding or 'oozing' include electrodesiccation or the use of chemical agents such as thrombin and fibrin sealant. Electrodesiccation has several disadvantages. It is time consuming, associated with adjacent thermal damage and potentially dangerous in the vicinity of vital structures such as the pelvic side-wall vessels and ureter. The haemostatic properties of thrombin and fibrin sealant have been well established. In the last decade, these two agents have been increasingly used to

control bleeding in general and thoracic surgery (Spotnitz *et al.*, 1987; Kram *et al.*, 1990). However, there remains a concern that these agents may enhance post-operative adhesion formation in the peritoneal cavity (Gauwerky *et al.*, 1990). This randomized animal study was designed to test this assumption by investigating whether thrombin, when used to achieve haemostasis in an oozing model, caused more post-operative adhesions than electromicrosurgical desiccation. We also compared the various histological parameters of inflammation with both modalities of treatment.

### Materials and methods

Thirty sexually mature, white, female New Zealand white rabbits that weighed between 3.0 and 4.6 kg were used as the animal model. The animals were housed in individual cages and fed a standard diet.

Anaesthesia was induced with ketamine hydrochloride (Ketalar; Eczacıbasi AS, Istanbul, Turkey) 35 mg/kg i.m. body weight and xylazine hydrochloride (Rompun; Bayer, Istanbul, Turkey) 5 mg/kg i.m. body weight. A small area of the abdomen to accommodate the incision was shaved. Loose hair was aspirated with a hand held vacuum and the area further cleansed of hair by using adhesive tape. The shaved area was prepared with betadine solution. A sterile drape was then applied over the prepared area. The animals underwent laparotomy via a midline 3.5 cm incision. The uterine horns were exposed and an operating microscope was brought on to the surgical field to carry out the remainder of the procedure. In each animal, the right uterine horn was subjected to a measured injury. The measured injury consisted of 12 equidistant cuts made with microscissors at right angles to the antimesometrial surface of the horn, over a distance of 2 cm, starting 1.5 cm from the bifurcation. Each cut included the serosa and muscularis of the uterine horn but did not extend to the mucosa and induced sufficient oozing of blood. After the injury was effected, the animals were randomized to haemostasis with either electromicrodesiccation or thrombin solution. Microbipolar forceps were used for haemostasis in the electromicrosurgical group. In the thrombin group, 1 ml thrombin solution (100 US units), prepared by dilution of 1000 US units of thrombin powder (Thrombin Topical USP Thrombogen; Johnson & Johnson Medical Inc., Arlington, TX, USA) in 10 ml of saline, was applied to the injury using a tuberculin syringe.

In the first phase of the study, the histological changes of the acute injury and haemostasis with electromicrosurgery or thrombin solution were evaluated in two animals in each group. Owing to the fact that this would measure only the acute injury, both horns (four horns for each group) were used and submitted to the standard injury. Once the haemostasis was established, the injured horn sections were removed, fixed in formalin and submitted to histological evaluation.

In the second phase of the study comprising 26 animals, the longer-term effects of adhesion formation and histological parameters of inflammation were studied. In these 26 animals, only the right uterine horn was submitted to the standard injury as described above and the

**Table I.** The mean  $\pm$  SD of various histological parameters of inflammation following injury and haemostasis with the use of thrombin or electromicrodesiccation. All are non-significant

	Congestion	Fibrin deposition	Fibroblastic activity
Thrombin acute injury	1.5 $\pm$ 1.0	0.8 $\pm$ 0.6	0
day 2	1.5 $\pm$ 0.7	1.0 $\pm$ 0.8	0.5 $\pm$ 0.4
day 7	2.0 $\pm$ 1.1	1.5 $\pm$ 0.8	1.5 $\pm$ 0.9
Electromicrodesiccation acute injury	2.0 $\pm$ 0.8	1.0 $\pm$ 0.7	0
day 2	2.0 $\pm$ 0	2.5 $\pm$ 0.7	1.0 $\pm$ 0.6
day 7	2.3 $\pm$ 0.9	1.3 $\pm$ 0.8	1.3 $\pm$ 0.9

intact left horn served as control for each animal. After this initial intervention, one, two and 10 animals, in each group, were submitted to second-look laparotomy on post-operative days 2, 7 and 15, respectively. Second-look laparotomy was carried out in the same manner as the initial laparotomy. Once the peritoneal cavity was entered, the type and extent of adhesions were quantified. The type of adhesions was assigned a score. The scores for filmy, moderately dense and dense-vascular adhesions were 1, 2, and 3, respectively. To quantify the extent of adhesions, microsurgical adhesiolysis was performed using the microscissors under the operating microscope. The surface area involved in the adhesive process was measured in mm<sup>2</sup> as previously reported (Filmar *et al.*, 1986). In short, a piece of millimetric acetate was placed on the surface of the uterine horn, and affected areas were traced with a fine-tipped marker. The total surface area involved in adhesions was expressed in square millimetres.

The histological parameters of inflammation evaluated were congestion, fibrin deposition and fibroblastic activity. Each parameter was assigned a score from 0 to 3, 0 for none and 1, 2, and 3 for mild, moderate and extensive, respectively.

Data were expressed as mean  $\pm$  SD. Mann-Whitney *U* test was used for statistical analysis.

## Results

The mean congestion and fibrin deposition scores of acute injury and haemostasis with electromicrosurgery or thrombin solution were comparable ( $P > 0.05$ ) (Table I). As expected, no fibroblastic activity was noted at this stage.

In the second phase of the study, the mean congestion, fibrin deposition and fibroblastic activity scores were comparable on days 2 and 7 ( $P > 0.05$ ) (Table I).

The surface area involved in adhesions as assessed by second-look laparotomy on day 15 was comparable between the electromicrosurgery (53.1  $\pm$  56.7 mm<sup>2</sup>) and thrombin (75.0  $\pm$  50.0 mm<sup>2</sup>) groups ( $P > 0.05$ ). The mean scores of the grade of adhesions were 1.2  $\pm$  1.1 and 2.0  $\pm$  1.1 in the electromicrosurgery and thrombin groups, respectively ( $P > 0.05$ ). None of the control horns in 26 animals had adhesions.

## Discussion

Oozing is not uncommonly encountered during various gynaecological surgical procedures. While individual bleeding vessels may be ligatured or desiccated, it may prove difficult to achieve haemostasis in the face of generalized oozing.

Electrodesiccation or agents, such as thrombin and fibrin sealant, may be used to control such generalized oozing. Electrodesiccation is time consuming, associated with adjacent thermal damage and may be potentially dangerous when used in the vicinity of vital structures, e.g. pelvic side-wall vessels and ureter. Local application of thrombin and fibrin sealant to control bleeding has recently gained popularity especially in general and thoracic surgery. However, adhesion formation remains a concern with their use in the peritoneal cavity.

Our injury model was judged to produce sufficient oozing. Thrombin solution was noted to control the oozing shortly after its local application in all cases. The extent and type of post-operative adhesions, as assessed by second-look laparotomy on day 15, were comparable between the thrombin and electromicrodesiccation groups. The mean scores of all three histological parameters of inflammation were also comparable between the two groups in both the acute phase and on post-operative days 2 and 7. Thrombin, therefore, was neither associated with increased adhesion formation nor worsened the histological inflammation parameters when compared to electromicrodesiccation.

Controversy remains on the effect of thrombin and fibrin sealant on adhesion formation. While some studies report more extensive adhesion formation with the use of fibrin sealant (Gauwerky *et al.*, 1990), others demonstrate no increase (McGaw *et al.*, 1988; Bruel *et al.*, 1993; Bilgin *et al.*, 1995; Evrard *et al.*, 1996; Marana *et al.*, 1996) and even a reduction (Lindenberg *et al.*, 1985; de Virgilio *et al.*, 1990; Tulandi, 1991; Caballero and Tulandi, 1992; Sheppard *et al.*, 1993; De Iaco *et al.*, 1994; Takeuchi *et al.*, 1996). The diversity of results may be due to differences in study design, injury model, control group, chemical composition and mode of application of the fibrin sealant and evaluation of adhesions.

There is paucity of data on the effect of thrombin on adhesion formation and histological parameters of inflammation. Wiseman *et al.* (1992), in a rabbit model, evaluated the effectiveness of thrombin in achieving haemostasis and its effect on adhesion formation. The authors also tested whether prior local application of thrombin solution affected the effect of oxidized, regenerated cellulose, TC7 (Interceed® Absorbable Adhesion Barrier; Ethicon Inc., Somerville, NJ, USA). To assess these parameters, the authors used both oozing and bleeding models. For the oozing model, a no. 10 scalpel blade was used to scrape both sides of each uterine horn  $\times 20$  along a 5 cm length to produce sufficient punctuate bleeding. For the bleeding model, four blood vessels on each uterine ligament were nicked to produce a heavier degree of bleeding. Following injury, a total of 139 rabbits were randomized to six groups. These included (1) control (oozing or bleeding was allowed to stop spontaneously), (2) thrombin only, (3) Interceed® only, (4) thrombin induced haemostasis followed by application of Interceed®, (5) thrombin induced haemostasis followed by Interceed® moistened with heparin and, finally, (6) thrombin soaked into Interceed® barrier before attaining haemostasis. Of these six groups, only the fourth and fifth groups had less adhesions when compared to the controls in both oozing and bleeding models. Group 2 (local application of thrombin

solution) was associated with an extent of adhesion formation similar to the controls.

Diffuse oozing of the operative site is not uncommon in abdominal and pelvic surgery. The pelvic side-wall is prone to this condition. Electromicrodesiccation in such circumstances is time consuming and may be hazardous because of its thermal effects and potential damage to the adjacent structures. Although thrombin is quite effective in controlling the condition, there has been reluctance to use the product, for fear that it may be adhesiogenic. The present study and the study of Wiseman *et al.* (1992) using the rabbit model demonstrate that the use of thrombin is not associated with a statistically greater degree of post-operative adhesions when compared to the use of electromicrodesiccation. However, we should stress that our study is preliminary and the sample size is limited. In other words, the absence of significant difference in the extent and type of adhesion formation as well as various histological parameters of inflammation between the electromicrodesiccation and thrombin groups may be due to a type II error. Further powerful prospective randomized trials, especially in human subjects, are required further to elucidate this issue.

#### Acknowledgements

We acknowledge Johnson & Johnson Medical Inc. for their generous supply of the thrombin powder.

#### References

- Bilgin, T., Cengiz, C. and Demir, U. (1995) Postoperative adhesion formation following ovarian reconstruction with fibrin glue in the rabbit. *Gynecol. Obstet. Invest.*, **39**, 186–187.
- Bruel, D., Gadonneix, P., Tranbaloc, P. *et al.* (1993) Comparison of different types of ovarian wound closure in rats. Role of biological glue. *J. Gynecol. Obstet. Biol. Reprod.*, **22**, 459–462.
- Caballero, J. and Tulandi, T. (1992) Effects of Ringer's lactate and fibrin glue on postsurgical adhesions. *J. Reprod. Med.*, **37**, 141–143.
- De Iaco, P., Costa, A., Mazzoleni, G. *et al.* (1994) Fibrin sealant in laparoscopic adhesion prevention in the rabbit uterine horn model. *Fertil. Steril.*, **62**, 400–404.
- de Virgilio, C., Dubrow, T., Sheppard, B.B. *et al.* (1990) Fibrin glue inhibits intra-abdominal adhesion formation. *Arch. Surg.*, **125**, 1378–1382.
- Evrard, V.A., De Bellis, A., Boeckx, W. *et al.* (1996) Peritoneal healing after fibrin glue application: a comparative study in a rat model. *Hum. Reprod.*, **11**, 1877–1880.
- Filmar, S., Gomel, V. and McComb, P. (1986) The effectiveness of CO<sub>2</sub> laser and electromicrosurgery in adhesiolysis: A comparative study. *Fertil. Steril.*, **45**, 407–411.
- Gauwerky, J.F.H., Mann, J. and Bastert, G. (1990) The effect of fibrin glue and peritoneal grafts in the prevention of intraperitoneal adhesions. *Arch. Gynecol. Obstet.*, **247**, 161–166.
- Kram, H.B., Tirso, J., Clark, S.R. *et al.* (1990) Techniques of splenic preservation using fibrin glue. *J. Trauma*, **30**, 97–101.
- Lindenberg, S., Steentoft, P., Sorensen, S.S. *et al.* (1985) Studies on the prevention of intraperitoneal adhesion formation by fibrin sealant. *Acta Chir. Scand.*, **151**, 525–527.
- Marana, R., Muzii, L., Catalano, G.F. *et al.* (1996) Use of fibrin sealant for reproductive surgery: a randomized study in the rabbit model. *Gynecol. Obstet. Invest.*, **41**, 199–202.
- McGaw, T., Elkins, T.E., DeLancey, J.O.L. *et al.* (1988) Assessment of intraperitoneal adhesion formation in a rat model: can a procoagulant substance prevent adhesions? *Obstet. Gynecol.*, **71**, 774–778.
- Sheppard, B.B., De Virgilio, C., Bleiweis, M. *et al.* (1993) Inhibition of intra-abdominal adhesions: fibrin glue in a long-term model. *Amer. Surgeon*, **59**, 786–790.
- Spotnitz, W.D., Dalton, M.S., Baker, J.W. *et al.* (1987) Reduction of perioperative hemorrhage by anterior mediastinal spray application of fibrin glue during cardiac operations. *Ann. Thor. Surg.*, **44**, 529–531.
- Takeuchi, H., Awaji, M., Hashimoto, M. *et al.* (1996) Reduction of adhesions with fibrin glue after laparoscopic excision of large ovarian endometriomas. *J. Amer. Assoc. Gynecol. Laparoscopists*, **3**, 575–579.
- Tulandi, T. (1991) Effects of fibrin sealant on tubal anastomosis and adhesion formation. *Fertil. Steril.*, **56**, 136–138.
- Wiseman, D.M., Gottlick, L.E. and Diamond, M.P. (1992) Hemostasis at a bleeding site using thrombin: efficacy of Interceed® (TC7) Absorbable Adhesion Barrier with and without heparin. *J. Reprod. Med.*, **37**, 766–770.

Received on January 9, 1998; accepted on February 27, 1998