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Effectiveness of auto-cross-linked hyaluronic acid gel in the prevention of intrauterine adhesions after hysteroscopic adhesiolysis: a prospective, randomized, controlled study

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BACKGROUND: A prospective, randomized, controlled study was performed to assess the efficacy of auto-cross-linked hyaluronic acid (ACP) gel in preventing the development of intrauterine adhesions following hysteroscopic adhesiolysis. METHODS: Ninety-two patients with irregular menses and intrauterine adhesions referred to the Hysteroscopic Unit of the University of Naples "Federico II". Patients were randomized to two different groups. Group A were randomized to hysteroscopic adhesiolysis plus intrauterine application of ACP gel (10 ml) and group B were randomized to operative hysteroscopy alone (control group). Baseline adhesion scores were calculated for each patient and at 3 months after surgery. RESULTS: Group A showed a significant decrease in intrauterine adhesions at 3 months follow-up in comparison with the control group. Staging of adhesions showed a significant decrease in adhesion severity in patients treated with ACP gel. CONCLUSIONS: ACP gel significantly reduces the development of intrauterine adhesions postoperatively and its use is likely to be associated with a reduction of severe adhesions.

Key words: adhesion score/hyaluronic acid gel/intrauterine adhesions/operative hysteroscopy

Introduction

Intrauterine adhesions are a recognized complication of operative hysteroscopy. Their development may result in infertility, recurrent miscarriages, irregular periods with dysmenorrhoea and pelvic pain (Valle and Sciarra, 1988). The most frequent cause of their formation is post-partum or post-abortion overzealous dilatation and curettage (Pabuçcu *et al.*, 1997).

In recent years, many attempts have been made to develop effective strategies to reduce the risk of post-surgical adhesions (Risberg *et al.*, 1997; Farquhar *et al.*, 2002; Watson *et al.*, 2002). Several in-vitro and in-vivo studies have demonstrated the efficacy of different barrier agents for preventing adhesions after laparotomy and laparoscopic gynaecological surgery (Mais *et al.*, 1995; Burns *et al.*, 1995; De Iaco *et al.*, 1998; Diamond *et al.*, 1998; Ferland *et al.*, 2001; di Zerega *et al.*, 2002). At the present time, few studies evaluating the efficacy of barrier methods for the prevention of intrauterine adhesions are available. For example, the insertion of an intrauterine device (IUD) following lysis of adhesions has been advocated by many authors as an effective method to prevent adhesions reformation, although the specific type to be utilized for this purpose remains a controversial issue. Other authors prefer the use of a ballon catheter, such as the Foley catheter (Schenker and Margalioth, 1982). Success in the safe enhancement of rapid endometrial growth has been reported with administration of estrogens or of a combination of estrogens and progestins, although the necessity for either has been queried (Schenker and Margalioth, 1982).

In recent years, hyaluronic acid (HA), a natural component of the extracellular matrix, the vitreous humor and synovial fluid of the joint, has been proposed as a barrier agent to prevent adhesion development after surgery (Burns *et al.*, 1995).

A new barrier agent containing an HA derivative, the Seprafilm membrane, has recently been described for the prevention of post-surgical adhesions. Seprafilm is a novel bioresorbable membrane formulated from chemically modified HA and carboxymethyl cellulose. It has been proposed as an effective adjuvant in reducing the incidence, extent and severity of abdominal and pelvic post-surgical adhesions (Becker *et al.*, 1996). Seprafilm has also been suggested as an effective method to reduce the presence and extent of intrauterine adhesions (Tsapanos *et al.*, 2002).

Table I. Patients' characteristics

Characteristic	Group A (ACP gel) (n = 43)	Group B (control) (n = 41)	Significance
Age (years) (± SD)	29.8 ± 4.1	30.7 ± 2.6	NS
Weight (kg) (means ± SD)	64.4 ± 4.6	62.8 ± 4.4	NS
Uterine size (hysterometry) (cm) (means ± SD)	6.9 ± 1.2	6.6 ± 1.5	NS
Parity	1.3 ± 0.2	1.5 ± 0.1	NS
Number of infertile patients	18	16	NS

NS = not significant.

Previous experimental preclinical studies have shown that cross-linked HA reduces adhesion formation after abdominal and pelvic surgery (Burns *et al.*, 1995; Thornton *et al.*, 1998; Johns *et al.*, 2001). HA has been modified to obtain an auto-cross-linked HA (ACP) gel (De Iaco *et al.*, 1998; 2001) that seems particularly suitable for preventing adhesion formation because of its higher adhesivity and more prolonged residence time on the injured surface than unmodified HA (Mensitieri *et al.*, 1996).

The aim of this prospective, randomized, controlled study was (i) to assess the efficacy of the ACP gel in the reduction of post-surgical adhesions reformation in women undergone hysteroscopic adhesiolysis, and (ii) to evaluate the characteristics of the adhesions observed at follow-up.

Materials and methods

The protocol of the study was approved by our Institutional Review Board and the study was conducted according to the guidelines of the 1975 Declaration of Helsinki on human experimentation.

All patients with intrauterine adhesions at diagnostic hysteroscopy were invited to participate in the study. From June 2001 to September 2002, 92 women (mean age ± SD, 30.1 ± 3.5 years) were enrolled in the study.

The inclusion criterion was hysteroscopic diagnosis of intrauterine adhesions. Exclusion criteria were: age >50 years, weight >100 kg, menopause (FSH >40 mIU/ml, 17β-estradiol <20 pg/ml) or pregnancy (positive β-hCG test), presence of uterovaginal prolapse and severe urinary symptoms, presence of malignancy, presence of severe intercurrent illness (coagulative disorders, systemic disease, severe cardiopathy), presence of other intrauterine lesions (i.e. polyps, myomata, septa).

Before entering the study, the purpose of the protocol was explained clearly to women attending our Hysteroscopic Unit, and a printed explanatory consent form was signed and obtained by all subjects enrolled.

Diagnostic hysteroscopy was performed using a 3.5 mm instrument (Gynecare Versascope; Gynecare, Ethicon Inc., Somerville, NJ, USA) using normal saline solution (NaCl 0.9 %) as the distension medium. Before hysteroscopy, all patients underwent vaginal examination to ascertain the position and size of the uterus, and a speculum was inserted into the vagina to expose the cervix.

Following diagnostic hysteroscopy, patients were randomized into two groups: group A (n = 46), the treatment group, and group B (n = 46), the control group, using a computer-generated randomization list. The design of the study followed CONSORT guidelines (www.CONSORT-statement.org) and the patient flowchart is set out in Figure 1 of these guidelines.

The treatment group received an intrauterine application of 10 ml of ACP gel (Hyalobarrier gel; Baxter, Pisa, Italy) under hysteroscopic view after operative hysteroscopy. The only intervention performed in the control group was hysteroscopic resection of intrauterine adhesions.

Operative hysteroscopy was performed using a rigid resectoscope (Karl Storz, Tuttlingen, Germany) with a 12-degree fore-oblique telescope with a hook-shaped monopolar electrode.

In group A, ACP gel was introduced into the uterine cavity at the end of the procedure through the out-flow channel of the resectocope while the surgeon progressively limited the entering of the distension medium through the in-flow channel. The procedure was considered complete when, under hysteroscopic view, the gel seemed to have replaced all the liquid medium and the cavity appeared completely filled by the gel from tubal osthia to internal uterine orifice.

Intrauterine presence of ACP gel was confirmed over the time by postoperative ultrasound evaluation. Ultrasound scans were performed in each patient from group A immediately after ACP gel application and after 24, 48 and 72 h. The gel-related hyperechoic thickness that seemed to separate endometrial walls was the mean evaluated parameter.

Patients from both groups were administered oral antibiotics (cefixima 400 mg/day) (Cefixoral; Menarini, Firenze, Italy) for 3 days after surgery.

Each patient underwent a follow-up diagnostic hysteroscopy 3 months after the surgical procedure and their adhesion score was assessed.

Both the initial diagnostic hysteroscopy and the 3-month follow-up diagnostic hysteroscopy were performed by the same operator (G.A.). G.A. evaluated the adhesion score for each patient and was blind for patients' randomized allocation, whilst operative hysteroscopies and application of ACP gel were performed by a different operator (M.G.).

Statistical analysis was performed with the use of a commercial software program (Statistica for Windows; Statsoft, Inc., Tulsa, USA). Data distribution was performed using Shapiro–Wilks test. Differences in age, weight and parity, which showed a normal distribution, were compared using the two-tailed Student's t-test for unpaired data. Repeated analysis of variance (ANOVA) followed by the Newman–Keuls multiple range test are used to compare the adhesion score before and after surgery. The Wilcoxon sum rank test was used to compare adhesion scores at 3 months between group A and B and the χ^2-test was used for proportions. $P < 0.05$ was considered as statistically significant.

Results

Characteristics of the patients treated are reported in Table I. There were no significant differences in age, weight, body mass index, uterine size and parity between patients in groups A and B.

Ultrasonographic data showed that ACP gel was able to keep uterine walls separated for at least 72 h. The ACP gel-related hyperechoic thickness was 10 ± 1.2 mm immediately after the procedure, 7.9 ± 0.2 mm at 24 h, 5.2 ± 0.3 mm at 48 h and 2.7 ± 0.4 mm at 72 h.

At 3 months follow-up, a significantly lower rate of post-surgical intrauterine adhesions was observed in group A (six out of 43 patients) in comparison with group B (13 out of 41 women) (13.95% versus 31.70%; $P < 0.05$). Eight women (three from group A and five from group B) did not attend for follow-up hysteroscopy.

The mean adhesion score was significantly lower in both groups A and B at 3 months follow-up in comparison with the baseline adhesions score (group A: 2.0 ± 0.0 versus 6.2 ± 0.7, $P < 0.001$; group B: 5.3 ± 0.2 versus 6.3 ± 0.9, $P < 0.05$). Patients in group A showed significantly lower adhesions scores at follow-up in comparison with those in group B (2.0 ± 0.0 versus 5.3 ± 0.2, $P < 0.001$).

When intrauterine adhesion staging, performed according to the American Fertility Society (The American Fertility Society, 1988) was evaluated, patients from group A showed a significant decrease in adhesions severity (100% stage I, mild adhesions) in comparison with group B (25% stage I, mild adhesions; and 75% stage II, moderate adhesions).

No significant differences were observed in intrauterine adhesion localization at follow-up.

Discussion

Hysteroscopic surgery may result in significant intrauterine adhesions. These can be managed safely and effectively hysteroscopically, restoring fertility and normal menstrual patterns. However, intrauterine adhesions can reform even following surgical treatment (Pabuçcu *et al.*, 1997).

Several methods have been used to prevent post-surgical adhesions. They can be classified as either pharmacological agents (anti-inflammatory, antioxidants, anticoagulants and fibrinolytics) (Ar'Rajab *et al.*, 1991; Kappas *et al.*, 1992; Hellebrekers *et al.*, 2000) or physical barriers, where substances are interposed between adjacent injured surfaces to avoid direct contact after surgery (Mais *et al.*, 1995; Ferland *et al.*, 2001; di Zerega *et al.*, 2002). In recent years, several methods have been used to prevent intrauterine adhesions, as well as IUDs or the Foley catheter intrauterine insertion after surgery.

HA is a particularly promising substance. It is a natural component of the extracellular matrix, the vitreous humor and synovial fluid of the joint. The anti-adhesive effects depend on the molecular weight as well as the concentration of the preparation (De Iaco *et al.*, 1998).

Recently, Tsapanos *et al.* (2002) showed a significant reduction of intrauterine adhesions using Seprafilm, a bioresorbable membrane formulated from chemically modified HA (sodium hyaluronate) and carboxymethyl cellulose.

HA has been modified with numerous molecules to obtain ferric hyaluronate (Thornton *et al.*, 1998; Johns *et al.*, 2001), a hyaluronic-carboxymethylcellulose membrane (Vrijland *et al.*, 2002), and it has been prepared as an auto-cross-linked gel (De Iaco *et al.*, 1998). The advantage of auto-cross-linked HA gel is that it has an increased viscosity compared with uncross-linked HA (Mensitieri *et al.*, 1996). This high viscosity and adhesiveness makes it easier to introduce the gel into the uterine cavity. In our experience, ACP gel displaces the liquid distension medium during introduction and remains *in situ* for at least 72 h (data not shown). Animal data suggest that HA gel remains *in situ* for more than 5–6 days (Laurent and Fraser, 1992; Nimrod *et al.*, 1992).

In in-vivo preclinical studies ACP gel has been reported to significantly reduce the incidence and severity of adhesion (De Iaco *et al.*, 1998; Kocak *et al.*, 1999; Belluco *et al.*, 2001). In this study, we evaluated a new ACP gel preparation. This new HA derivative seems to be well tolerated (De Iaco *et al.*, 1998; Pellicano *et al.*, 2003).

The objective of this prospective study was to demonstrate the efficacy of ACP gel in the prevention of post-surgical adhesions reformation after hysteroscopic surgery and to evaluate the influence of this substance on the gravity of intrauterine adhesions formed after surgery.

Our randomized, controlled trial showed a significant reduction in post-operative intrauterine adhesions in the ACP group. The effect on long-term reproductive outcome is not clear but will emerge from our ongoing work. This early work suggests that ACP gel is a promising new absorbable agent barrier; however, the results should be confirmed in further studies before it is introduced into widespread clinical practice.

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