Adhesion preventive effect of hyaluronic acid after intraperitoneal surgery in mice

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Prevention of intraperitoneal adhesion after gynaecological surgery is essential for maintaining postoperative fertility. In this study, the adhesion prevention effect was examined of a hyaluronic acid (HA) solution obtained from the fermentation method and having a molecular weight of 1.9×10⁶ with high viscosity. Laparotomy was conducted on female mice 7 weeks old, whose menstrual periods were synchronized by pregnant mare serum gonadotrophin (PMSG) to injure the uterine horn surface. Intraperitoneal adhesions were favourably formed in 91.7% of cases induced with iodine abrasion, compared with 50% induced by electrosurgery. Intraperitoneal administration of HA was evaluated for its effect on the prevention of adhesions made by iodine abrasion. Adhesion prevention effects of HA were observed at concentrations of 0.3, 0.5, 0.75 and 1.0%, among which the most pronounced effect was with the use of a 0.3% solution (92.3% of cases). Compared with the control group adhesion score of 2.0 ± 0.8, significant decreases in adhesion scores were observed at all concentrations. HA with a molecular weight of 1.9×10⁶ was recognized to have a definitive prevention effect on postoperative adhesions in mice after laparotomy and is considered to be a prospective material for future clinical use.

Key words: adhesion/adhesion prevention/hyaluronic acid/murine uterine horn model/reproductive surgery

Introduction

Postoperative adhesion formation and reformation remains a serious problem in peritoneal surgery, especially in gynaecological reproductive surgery. Many studies on postoperative adhesion date from 20 years ago (Ellis, 1963; Raftery, 1973). It was considered to be important for adhesion prevention to maintain a moist state at the operative field using Ringer’s solution or physiological saline solution, along with avoiding contact with foreign bodies during the operation. However, despite using these microsurgical techniques, >50% of patients are thought to develop postoperative adhesions (Gomel, 1983; DeCherney and Mezer, 1984). Under these circumstances, adhesion preventive devices such as Interceed and Seprafilm® were investigated and found to be useful after gynaecological and general surgery. Although these barriers have clinical advantages for adhesion prevention, their benefits are limited to the site of placement of the membrane or film, and more effective adjuvants are desirable for this purpose.

Hyaluronic acid (HA) is a linear polysaccharide with repeating disaccharide units, naturally occurring from extracellular matrix components. Experimental and clinical data using various contents of HA solution have suggested that covering an operation field with soluble HA would reduce postoperative adhesions (Rogers et al., 1997; Thornton et al., 1998).

This report evaluates the efficacy of a soluble barrier fluid composed of high molecular weight HA, which is different from conventional HA in its molecular makeup, in reducing postoperative adhesion formation in the murine uterine horn model.

Materials and methods

Adhesion formation model

The first step was to determine the best method for provoking adhesion formation. Three methods were tried. After synchronizing the menstrual periods of 48 ICR female mice 7 weeks old with the intraperitoneal administration of 5 IU of pregnant mare serum gonadotrophin (PMSG), laparotomies were conducted 48 h later under anaesthesia with intraperitoneal injection of Nembutal sodium solution (Abbott Laboratories, North Chicago, IL, USA). After confirming that there was no sign of intraperitoneal infection, three types of trauma were inflicted on both uterine horns: (i) incision and suture: an incision was made on half the circumference of the uterine wall with a scalpel and the edges were re-joined with 5–0 vicryl three interrupted sutures; (ii) desiccation with electrosurgery: a length of 1.5 cm of the uterine horn was desiccated for 2 s at 3 W; and (iii) iodine abrasion: a 1.5 cm abrasion was made on uterine horns with a cotton ball permeated with iodine ethanol moved back and forwards four times. The control group (n = 24) had no trauma induced on either uterine horn except for opening the abdominal wall. A second look laparotomy was performed 7 days after the initial procedure, and adhesion formations on the wounded uterine surfaces between abdominal fat tissue and organs were evaluated. Those regions having >1 mm adhesions were considered positive for adhesions.

Examination of the adhesion prevention effect of HA

Materials

The high molecular weight HA was manufactured by Denkikagaku Kogyo Co. Ltd (Tokyo, Japan). It has a molecular weight of 1.9×10⁶ and is purified by the fermentation method; it is sterile and non-pyrogenic, with neutral pH, and is manufactured with a high intrinsic viscosity twice that of conventional HA.

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Methods

HA was administered into the abdominal cavity of 80 ICR mice of 7 weeks old. The HA was adjusted to concentrations of 0.3, 0.5, 0.75 and 1.0, and 3 ml of each was intraperitoneally injected into 13, 25, 31 and 11 mice respectively, immediately before closing the wound at the end of the surgery to induce adhesions (by the iodine abrasion method; see Results below). For the 25 control mice, 3 ml of physiological saline was injected under the same conditions. In order to examine the effect of different molecular weights of HA, 3 ml of sodium hyaluronate [HA-S: having molecular weight 0.6–1.2 × 10^6] and collected from rooster combs by extraction (Swann, 1968; Seikagaku Kogyo Co. Ltd, Tokyo, Japan) was injected into 11 mice in the same manner.

Concentrations of intraperitoneally administered HA were measured daily by high performance liquid chromatography (HPLC) using a column of Shodex OHpak KB806 with flow rate of 0.5 ml/min with 0.1 mol NaNO₃ (Beaty et al., 1985). This was used to examine the metabolism of HA in the abdominal cavity.

The adhesion prevention effects were determined at a second-look laparotomy on the 7th postoperative day. The adhesion scores were measured using a 0–3 point scale for the following four states: 0, no adhesion (−); 1, slight adhesion (1−); 2, moderate adhesion (2+); adhesion of ~1 mm; and 3, severe adhesion (3+); adhesion of >3 mm.

The adhesion suppressing effects were measured against the control group. A suppressing effect of 1 point or more on the 0–3 scale was interpreted as a preventive effect.

Statistical analysis was performed using the χ²-test where appropriate, Fisher’s exact test or Student’s t-test. A P value < 0.05 was considered statistically significant.

Results

Adhesion occurrence rate in the adhesion formation models

The adhesion occurrence rates at the second-look laparotomy were compared among the control group and three different trauma groups: incision and suture, electrosurgery and iodine abrasion. The adhesion occurrence rate was significantly higher in the iodine abrasion group (P < 0.02) (Table I). Since the iodine abrasion method was the most effective means of provoking adhesions, this method was therefore used to provoke adhesions for the main experiment.

Daily change of the intraperitoneal concentrations of HA

The concentration of intraperitoneally administered HA showed a daily decrease and at 96 h postoperatively (on day 4) was below the level of sensitivity by the HPLC measurement method. No HA was detected in the control group throughout the entire experimental period (Figure 1).

Adhesion prevention effect of HA

Postoperative adhesion decreasing effect of HA

The percentages of mice in which a decrease in postoperative adhesions was recognized when compared with the control group were 92.3, 76.0, 61.3 and 72.7% for the 0.3, 0.5, 0.75 and 1.0 HA groups respectively. Percentage reduction in the group with added HA-S was 45.5%. The difference between the 0.3% HA group and the HA-S group was significant (P < 0.02) (Table II).

Examination of the adhesion prevention effect of HA using the adhesion scores

The postoperative adhesion scores are shown in Table III. All the HA-added groups showed a significant decrease in adhesion score compared with the control group. However, there was no significant difference among the HA groups. On the other hand, the score in the HA-S-added group was 1.7 ± 0.5, which was significantly different from the 0.3% HA group (P < 0.03) but not from the control group.
Discussion

Adhesion prevention models are essential and important for determining the success of various methods of adhesion prevention. However, they have several problems when used in experimental animals, such as sex and age of model, or period of the menstrual cycle. In making the present model, uterine horns were used as the intraperitoneal organ and trauma was applied to the uterine horns for evaluating the preventive effect. In order to synchronize the menstrual periods for matching animal conditions (identical age in weeks), gonadotrophin was injected preoperatively before the experiment. In addition, because the scale of adhesion formation depends on the intensity of the trauma, a constant intensity of trauma was maintained throughout and adhesion length was used as the evaluation standard. On the other hand, it has been reported that animal models are not appropriate for determining the adhesion prevention effect in humans (Diamond, 1997). It is stated that in animal experiments, the organs are entirely normal before surgery, which is completely different from the situation in humans (pre-existence of adhesions) in terms of conditions; however, the animal model has the advantage of being able to set the same conditions for all experiments. Moreover, the experimental method differs greatly depending on the purpose: whether to prevent de-novo adhesion formation or to prevent adhesion reformation after adhesiolysis. The present study concerned the prevention effect on de-novo adhesions.

In the present study, three methods were tested to induce adhesion formation: suture, desiccation with electrosurgery, and iodine abrasion. Other reported adhesion forming methods include wounds made by CO2 laser (Urman and Gomel, 1991), and iodine abrasion. Other reported adhesion formation: suture, desiccation with electrosurgery, and abrasion using sandpaper (Shushan et al., 1994), all reported the prevention effects for de-novo adhesions. The method using iodine abrasion, which in our hands showed the best reproducibility and had a favourable adhesion formation rate, was used in the present adhesion prevention experiment.

The adhesion prevention effect of HA is clarified by our experiment, and it was found that lower concentrations were more effective. This result agrees with a report on the relationship between the viscosity of HA and its adhesion prevention effect (Burns et al., 1995), and with a report on the better effect of HA when used at lower viscosity and lower molecular weight (Rogers et al., 1997). On the other hand, it has been reported that 1% HA concentration is most effective (Shushan et al., 1994). The concentration and amount of HA to administer in future to human beings needs further experimentation, since the results obtained in this study were from mice.

The adhesion prevention mechanism of HA is still unclear and it is difficult to throw any more light on it from our present experiment. In another study, ferric hyaluronate gel with a half-life in the peritoneal cavity approximately twice that of conventional HA reduced significantly the number, severity and extent of adhesion formation in the abdominal cavity after laparotomy (Thornton et al., 1998). Considering the rapid decline of the intraperitoneal concentration of HA (lowered to normal value on the 4th day; half-life ~24 h), it can be concluded that the prevention mechanism is working at an extremely early stage of adhesion formation. Therefore, the co-operative adhesion suppression of fibroblasts and/or the platelet coagulation suppression can be assumed to be related to this mechanism (Shushan et al., 1994), in addition to the coating effect which was shown by the shallower tissue injury crater depth on histological examination after preoperative HA administration (Urman et al., 1995). The adhesion suppression mechanism is known to be associated with the appearance of various cytokines, such as epidermal growth factor or transforming growth factor-β, that suppress the activity of tissue repair cells in the postoperative period via macrophages (Fukazawa et al., 1989). Further research is needed to explain the mechanism fully.

Acknowledgements

The authors acknowledge Masamichi Hashimoto and co-workers in the Research Center of Denkikagaku Kogyo Co. Ltd. High molecular weight hyaluronic acid solutions were kindly provided by their manufacturers.

References


Received on November 11, 1998; accepted on February 24, 1999