Development of a novel glucose polymer solution (icodextrin) for adhesion prevention: pre-clinical studies

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Intra-abdominal adhesion formation causes significant post-operative morbidity. Controlled studies using animal models have been carried out to assess the tolerability and preventive efficacy of icodextrin solution (a biodegradable, biocompatible, glucose polymer). Reduction of adhesion formation was first evaluated in a rabbit double uterine horn model, applying 10–75 ml of 7.5 and 20%, or 50 ml of 2.5–20% icodextrin solution post-operatively. Significant increases in adhesion free sites (P < 0.005) were observed with volumes ≥25 ml, and at concentrations ≥4%. Efficacy of 50 ml 4 and 20% icodextrin was then evaluated both during and after surgery, demonstrating significant reductions in adhesion formation (P < 0.002). In one study, intraplus post-operative use of 4% icodextrin produced the greatest reduction of non-surgical site adhesions; in others, the post-operative effect was predominant. Post-surgical administration of 50 ml 4% icodextrin in a rabbit sidewall model also resulted in more adhesion-free animals, and a significant reduction (P < 0.001) in areas of adhesion formation and reformation. In a rat infection potentiation model, 4% icodextrin produced no difference in mortality, abscess formation or overall abscess score. These data suggest that 4% icodextrin offers a well-tolerated and effective means of reducing post-surgical adhesion formation.

Key words: glucose polymer/iodextrin/peritoneal adhesions/preclinical

Introduction

Peritoneal adhesions develop in a majority of subjects during the post-operative period, with as many as 80% of gynaecological procedures resulting in the formation of adhesions (diZerega and Rodgers, 1992a,b,c; diZerega, 1994; Monk et al., 1994). Adhesion formation after peritoneal surgery is a major cause of post-operative bowel obstruction, infertility and chronic pelvic pain (Miller and Winfield, 1959; Bronson and Wallach, 1977; Kresch et al., 1984; Stout et al., 1991; Howard, 1993; Strickler et al., 1994; Tulandi et al., 1998). Therefore, a method by which post-surgical adhesion (PSA) formation could be reduced or prevented would be of great benefit in reducing post-operative morbidity and mortality.

Two major epidemiological studies in general surgery from the USA and UK illustrate the considerable morbidity resulting from PSA formation (Beck et al., 1999; Ellis et al., 1999). They demonstrate the need for a simple, cost-effective means of reducing this morbidity. While the clinical and financial impact of complications due to adhesions is extensive, the reason for the lack of recognition of PSA formation is probably owing to the absence of a non-invasive means of quantifying adhesion formation in man (Holmdahl, 1999). Clinical studies in reduction of PSA formation have therefore been limited to procedures where second-look laparoscopy is justifiable.

Studies have indicated that placement of an absorbable barrier of oxidized regenerated cellulose (INTERCEED®), expanded polytetrafluoroethylene (Prelude®), or hyaluronic acid (HA)/carboxymethylcellulose (Seprafilm®) between injury sites or the addition of a viscous solution such as ionically cross-linked 0.5% HA (Intergel™), 32% dextran 70 (Hyskon®), or low viscosity 0.04% HA (Seprocoat®), into the peritoneal cavity during or after surgery can reduce post-operative adhesion formation (Azziz, 1993; Franklin et al., 1995; Mais et al., 1995; Nordic Adhesion Prevention Study Group, 1995; Keckstein et al., 1996; Johns et al., 1997; Thornton et al., 1998). In the case of site-specific adjuvants, the surgeon must predict potential sites of adhesion formation in order to determine placement and optimize barrier benefit. Low viscosity HA, which is not site specific, has been shown only to effectively reduce the number of de-novo adhesions at sites remote from the surgical trauma (Diamond et al., 1988), whilst no benefit was seen at the surgical sites. The use of dextran 70, an α, 1–6 linked dextrose polymer which is absorbed systemically but metabolized very slowly, produced undesirable local and systemic side-effects when used in clinical practice, resulting from its osmotic properties (Gauwerky et al., 1986; Trimbos-Kemper and Veering, 1989). In clinical trials an increase in post-operative serum glutamic oxalacetic transaminase (SGOT) and serum glutamic–pyruvic transaminase (SGPT) values and a transient increase in serum transaminase concentrations were reported, while other side-effects reported included vulvar oedema, pleural effusion, anaphylactic shock or allergic symptoms. Several other reports indicate that the 200–250 ml dextran 70 used is ineffective in pelvic surgery owing to gravitational pooling (Gauwerky et al., 1986).

To overcome the problems associated with dextran, and to overcome the clinical problems associated with site and non-site specific adhesions, an iso-osmolar biodegradable glucose

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polymer solution (Adept™) containing 4% icodextrin is being developed for use in intra-abdominal surgery. Icodextrin is an α, 1–4 linked glucose polymer produced by the hydrolysis of corn starch and fractionated by membrane separation technology to produce material with the desired molecular weight distribution. Icodextrin is a substrate for amylase, which is widely distributed throughout the body but is not present in the human peritoneal cavity (Davies, 1994). When given i.p., icodextrin is largely retained within the peritoneal cavity, absorption of the polymer occurring gradually via the lymphatic system into the systemic circulation. Icodextrin is then readily metabolized by amylase to oligosaccharides, which are cleared by further metabolism to glucose. Dextran, by comparison, is not a substrate for amylase and is only slowly metabolized on entry to the systemic circulation. The fluid dynamics of 4% icodextrin show that a volume placed into the peritoneal cavity after surgery would be resident during the time of maximum risk of adhesion formation, up to 3–5 days post-surgery (Raftery, 1973; diZerega and Rodgers, 1992a; Harris et al., 1995; Gilbert et al., 1999), and may provide a suitable means of keeping the tissue surfaces apart by flotation, providing a barrier to adhesion formation at both surgical site and non-site areas.

We have undertaken a programme of research to investigate the tolerability and efficacy of icodextrin solutions in the prevention of post-operative adhesions. We have evaluated the solution in two rabbit adhesion models; the double uterine horn (DUH) model and the rabbit sidewall model of adhesion formation and reformation. The effect of icodextrin on infection potentiation is presented in a rat model of bacterial peritonitis.

### Materials and methods

**Animals**

Female New Zealand White rabbits, 2.4–2.7 kg and female Sprague–Dawley rats, 175–225 g were purchased from Irish Farms (Norco, CA, USA) and quarantined in the University of Southern California vivaria (an accredited facility) for at least 2 days prior to use. All procedures were approved by the USC Institutional Animal Care and Use Committee and were well tolerated by the animals. The animals were randomized into treatment groups prior to initiation of surgery. Animals were housed on a 12 h:12 h light:dark cycle with food and water available ad libitum.

**Materials**

Concentrations of 2.5, 4, 7.5, 10, 15 and 20% (w/v) icodextrin solution, and placebo solution were supplied by ML Laboratories plc (Blaby, UK). The placebo electrolyte solution contained the same concentrations of sodium chloride, sodium lactate, calcium chloride and magnesium chloride as icodextrin solution, in 1 l of water for injection, but contained no icodextrin. The sutures used to close the

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**Table I. Summary of study treatments**

<table>
<thead>
<tr>
<th>Study 1: volume evaluation (13 groups)</th>
<th>Wash (pre- + intra- + post-surgical)</th>
<th>Instillate (post-surgical)</th>
<th>Surgical control (no wash/instillate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>10–75 ml of:</td>
<td>7.5% icodextrin or placebo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20% icodextrin or placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 2: concentration evaluation (9 groups)</td>
<td>No</td>
<td>50 ml of: 2.5–20% icodextrin placebo or RLS</td>
<td>Yes</td>
</tr>
<tr>
<td>Study 3: optimal solution (10 groups)</td>
<td>1st set, no</td>
<td>4% icodextrin RLS or saline</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>2nd set: 4% icodextrin</td>
<td>2nd set: 4% icodextrin RLS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20% icodextrin</td>
<td>saline RLS 4% icodextrin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>saline</td>
<td>3rd set 4% icodextrin saline</td>
<td></td>
</tr>
<tr>
<td>Study 3 extension (3 groups)</td>
<td>4th set: 4% icodextrin</td>
<td>4th set: 4% icodextrin</td>
<td></td>
</tr>
<tr>
<td>Rabbit sidewall model</td>
<td>No</td>
<td>50 ml 4% icodextrin continued to week 2</td>
<td>Yes (n = 19)</td>
</tr>
<tr>
<td>Adhesion formation (week 1)</td>
<td>(n = 13)</td>
<td>50 ml 4% icodextrin continued to week 2</td>
<td>Yes (n = 10)</td>
</tr>
<tr>
<td>Reformation (week 2)</td>
<td>No</td>
<td>20 mg/kg of: 4% icodextrin or RLS</td>
<td>Yes</td>
</tr>
<tr>
<td>Rat infection potentiation</td>
<td></td>
<td>20 mg/kg of: 4% icodextrin or RLS</td>
<td>Yes</td>
</tr>
<tr>
<td>LD10 (n = 45)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD50 (n = 45)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RLS = Ringer’s lactated solution.
muscle and skin were 3–0 coated Dexon II suture for rabbits (Davis and Geck, Manati, PR, USA) and 4–0 Ethilon (Ethicon, Somerville, NJ, USA) for rats.

In this series of experiments, three animal models were used (see Table I for summary): the rabbit double uterine horn model; the sidewall model of adhesion formation and reformation in rabbits; and the rat model of infection potentiation (Weinstein et al., 1974). In each study, 10 animals were assigned per group unless otherwise stated.

**Double uterine horn model (DUH)**

Rabbits were anaesthetized with a mixture of 55 mg/kg ketamine hydrochloride and 5 mg/kg Rompum i.m. Following preparation for sterile surgery, a midline laparotomy was performed. The uterine horns were exteriorized and traumatized by abrasion of the serosal surface with gauze until punctate bleeding developed (Nishimura et al., 1984; Rodgers et al., 1996, 1997a,b, 1998a,b). Ischaemia of both uterine horns was induced by removal of the collateral blood supply. The remaining blood supply to the uterine horns was the ascending branches of the utero-vaginal arteries.

**Study 1: volume evaluation**

At the end of surgery 10, 15, 25, 50 or 75 ml of 7.5 or 20% icodextrin solutions, or 10 or 75 ml placebo solutions were instilled prior to closure of the abdomen. In control animals there was no instillate.

**Study 2: concentration evaluation**

Aliquots of 50 ml of various concentrations of icodextrin solution (2.5, 4, 7.5, 10, 15 or 20%), 50 ml placebo solution or 50 ml Ringer’s lactated solution (RLS) were instilled at the end of surgery. In control animals there was no instillate.

**Study 3: intra-operative washing and post-operative instillation of icodextrin**

Using an optimal concentration of the solution, as determined in study 2 (4% icodextrin), the efficacy of icodextrin used as an intraoperative wash and as a post-operative instillate was evaluated. This was compared with the use of saline, RLS and surgical controls, and with the highest concentration available (20% icodextrin). The 20% icodextrin group was included to provide confirmation that the 4% solution was equally as effective as higher concentrations.

In this study, 30 ml of solution was administered pre-operatively and removed by aspiration before initiation of surgery, 20 ml of solution was administered to the site of surgery four times intra-operatively (5 ml at each administration) and 30 ml of solution was administered and aspirated at the end of surgery as a post-operative wash. A further 50 ml of solution was instilled into, and left in the cavity at the end of surgery.

For this study there was one surgical control group (receiving no treatment during or after surgery), three groups of instillate only (4% icodextrin, RLS, and saline) with no intra-operative use of solutions for washing, four groups where the same solution was used intra- and post-operatively (4% icodextrin, 20% icodextrin, RLS and saline) and two groups where either RLS or saline was used intra-operatively with post-operative instillation of 4% icodextrin.

Study 3 was extended to evaluate the efficacy of intra-operative washing only with 50 ml of 4% icodextrin (without the final instillate) compared with a surgical control and a 4% icodextrin wash + instillate group (Table I)

**Methods of scoring the adhesions**

All animals in these studies were killed on day 7 for evaluation of adhesions. Incidence of adhesions was determined by calculating the number of sites with/without adhesions present. Eight sites were evaluated per animal: four surgical and four remote, non-surgical sites. The percentage of the area of the uterine horns adherent to various organs was determined, and the tenacity of the adhesions was scored using the following system: 0 = no adhesions; 1 = mild, easily dissectable adhesions; 2 = moderate adhesions; non-dissectable, does not tear the organ; 3 = dense adhesions; non-dissectable, tears organ when removed.

An overall score based on the above assessments was assigned to each rabbit. The following scoring system was used: 0 = no adhesions; 0.5+ = light, filmy pelvic adhesions involving only one organ, typically only one or two small adhesions; 1.0+ = light, filmy adhesions, not extensive although slightly more extensive than 0.5; 1.5+ = adhesions slightly tougher and more extensive than a 1 rating; 2.0+ = tougher adhesions, a little more extensive, uterine horns usually have adhesions to both bowel and bladder; 2.5+ = same as 2, except the adhesions are usually not filmy at any site and more extensive; 3.0+ = tougher adhesions than 2, more extensive, both horns are attached to the bowel and bladder, some movement of the uterus possible; 3.5+ = same as 3, but adhesions slightly more extensive and tougher; 4.0+ = severe adhesions, both horns attached to the bowel and bladder, unable to move the uterus without tearing the adhesions.

The adhesions were scored by two independent observers who were blinded to the treatment of the animal. If there was disagreement as to the score to be assigned to an individual animal, the higher score was given. Overall scores were ranked prior to analysis.

**Sidewall formation and reformation model**

Rabbits were anaesthetized as before. Following preparation for sterile surgery, a midline laparotomy was performed. The caecum and bowel were traumatized and abraded to create subserosal haemorrhages and punctate bleeding over all surfaces. A 5 cm×3 cm area of peritoneum and transversus muscle was excised and abraded with gauze. The skin and the abdominal muscle were closed. Rabbits were randomized to receive either 50 ml of 4% icodextrin (n = 13) or no instillate (n = 19). One week later, the animals in the icodextrin group were killed and adhesions scored. The remaining animals were anaesthetized as described above and received a second laparotomy. Where adhesions were present, they were scored and lysed using blunt and sharp dissection. Care was taken not to injure the bowel (Rodgers et al., 1998a,b). After lysis of adhesions, the muscle incision was closed and 50 ml of 4% icodextrin was administered i.p. through the incision suture line prior to closure of the last suture in nine of the animals. Ten animals received no instillate, serving as reformation controls.

Seven days after surgery the rabbits were killed and the percentage of the area of the sidewall injury that was involved in adhesions was determined. Tenacity of the adhesions was scored as before using the 0–3 scale. In this model, a reduction in either the area or the tenacity of the adhesion was considered to be beneficial.

**Statistical analysis**

From the DUH model studies, the tenacity score and the overall scores were analysed by rank order analysis and by analysis of variance on the ranks. The percentage area of the horns involved to the various organs was compared by Student’s t-test.

In the sidewall and adhesiolysis model the percentage area of the sidewall involved to the various organs was compared using Student’s t-test and the incidence of adhesion-free animals (number of animals with no adhesions at all) was analysed using the χ² test.

**Infection potentiation model**

As icodextrin is a polymer with a carbohydrate backbone, the effect of icodextrin on bacterial peritonitis after inoculation with sufficient bacteria to cause 0–20% (LD₁₀₀) or 40–60% (LD₅₀) mortality in control animals was assessed (Weinstein, 1974).
Preparation of gelatin capsules

The caecal contents and faeces from rats fed hamburger for 2 weeks were collected and mixed 1:1 with non-sterile peptone yeast glucose broth containing no preservatives (Scott Laboratories, Arcadia, CA, USA) and 10% barium sulphate. The amount of this faecal preparation that caused mortality in 0–20% of the rats (LD_{10}) or 40–60% of the rats (LD_{50}) was determined. The appropriate amount of material to induce LD_{10} or LD_{50} was aseptically added to a gelatin capsule (Number 1; Eli Lilly Company, Indianapolis, IN, USA). This capsule was then placed in a second larger capsule (Number 00, Eli Lilly), and was referred to as a double-walled gelatin capsule. The capsules were prepared 3 days prior to implantation and stored in frozen conditions under quarantine until the day of surgery. The capsules were brought to room temperature prior to implantation.

Implantation of gelatin capsule

The rats (n = 90: 45 for LD_{10} study, 45 for LD_{50} study) underwent a standardized preparation for laparotomy (i.m. anaesthesia with ketamine/Rompum, shaving with animal clippers, betadine scrub, alcohol scrub). A 2 cm incision was made on the midline. A double-walled gelatin capsule was placed on the right side of the abdomen through the incision. In the control animals, no further treatment was given. In the treatment groups, 20 ml/kg of 4% icodextrin solution or RLS was placed on the left side of the abdomen between the visceral and parietal peritoneum.

The abdominal wall and skin were sutured closed using two layers of 4–0 Ethilon suture. Rats received analgesia (10–20 μg/kg bupronex) for 3 days and were observed twice daily for signs of morbidity/mortality.

Necropsy

Rats that died during the 11 day post-operative observation period were necropsied to confirm the presence of an acute bacterial infection. Rats surviving the initial acute infection were killed on day 11 after surgery. Each rat was examined for any abdominal abscesses by palpation through the skin, odour upon opening and splenomegaly. In addition, four sites of the peritoneum were examined for abscesses by palpation through the skin, odour upon opening and splenomegaly.

Results

Double uterine horn model studies

Study 1: volume evaluation

During post-operative evaluation, it was noted that 11 rabbits given the higher volumes of icodextrin had bulging abdomens (three rabbits with 75 ml 7.5% icodextrin up to 24 h and eight rabbits with 75 ml 20% icodextrin up to 72 h), but no leaking of fluid from the abdominal wound was recorded. This bulging was not observed in the rabbits treated with 75 ml placebo. No excess fluid was observed in any group at necropsy on day 7. One rabbit (75 ml 20% icodextrin) had a small fluid at necropsy. This was considered unrelated to the icodextrin treatment.

The effect of icodextrin on the formation of adhesions in this rabbit model can be found in Figure 1 (rank order of overall score) and Figure 2 (incidence of adhesions). At higher volumes (25–75 ml) of icodextrin, there was a significant reduction in the formation of adhesions when compared with either placebo (P < 0.001) or surgical controls (P < 0.001). However, no significant difference between the 7.5 and 20% solutions was noted in this study. No inflammation in excess of that expected from the surgical procedure was noted at necropsy. A 50 ml aliquot of icodextrin solution (~20 ml/kg)
Figure 3. Rabbit DUH model. Study 2: rank order of overall score for different concentrations of icodextrin (mean ± SEM). (Control = surgical control; RLS = Ringer’s lactated solution; placebo = diluent without icodextrin.) **P < 0.01; bP < 0.001 versus surgical control; cP < 0.01 versus RLS; dP < 0.05 versus placebo.

Figure 4. Rabbit DUH model. Study 2: incidence of adhesion formation for different concentrations of icodextrin, expressed as percentage of total sites that are adhesion free after treatment. (Control = surgical control; RLS = Ringer’s lactated solution; placebo = diluent without icodextrin.) *P ≤ 0.05 a versus both surgical and RLS controls; bversus placebo.

was selected as the optimal volume, as this volume did not produce bulging.

Study 2: concentration evaluation
Administration of 50 ml of icodextrin in various concentrations (2.5, 4, 7.5, 10, 15 and 20%) was shown to reduce the extent, tenacity and incidence of adhesion formation (rank scores Figure 3, and incidence Figure 4) (P < 0.05). The efficacy of 2.5% icodextrin was lower compared with the solutions containing a concentration of 4% icodextrin or more. As before, no excess fluid was present at necropsy and no inflammation was associated with the administration of icodextrin. In one, two and 10 rabbits that received 10, 15 and 20% icodextrin respectively, bulging of the abdomen was observed for up to 48 h after administration of the solutions. The 4% concentration of icodextrin (50 ml) was selected for subsequent studies.

Study 3: intra-operative washing and post-operative instillation of icodextrin
A post-operative instillate of 50 ml 4% icodextrin, as before, significantly reduced the extent, severity and incidence of adhesion formation in the DUH model when compared to the surgical control group (P < 0.001) and to the groups where RLS or saline were used (P < 0.01) (Figures 5 and 6). An instillate of either RLS or saline did not significantly reduce any adhesion parameter scored when compared to the surgical controls. This confirms the findings in study 2.

When icodextrin was used as an intra-operative wash with an instillate of the same solution post-surgery (using both 4 and 20% icodextrin) a significant reduction in the extent, tenacity and incidence of adhesions was demonstrated (P < 0.001) compared to surgical controls. There was no significant difference between the groups treated with 4% compared with 20% icodextrin. There was no significant difference between the 4% icodextrin instillate only group and the group treated...
with 4% icodextrin used as an intra-operative wash plus instillate (Figures 5 and 6). When surgical and non-surgical sites were viewed separately, however (Verco et al., 1999), the remote sites showed a trend in favour of 4% icodextrin wash plus instillate (67% of non-surgical sites were adhesion-free, compared with 55% in the instillate only group).

In the groups where the intra-operative wash was performed with either RLS or saline, with a post-operative instillate of 4% icodextrin, there was also a significant reduction in adhesion formation ($P < 0.001$) compared to controls. However, a similar reduction in adhesion formation was not observed after administration of saline or RLS as an intra-operative wash with the same solution as the post-operative instillate. When these two groups were compared to the groups where either 4 or 20% icodextrin was used intra- and post-operatively, the icodextrin groups had significantly lower overall adhesion scores ($P < 0.002$) (Figures 5 and 6). No excess fluid was present at necropsy and no inflammation was associated with the administration of icodextrin.

When 4% icodextrin was used for intra-operative washes without an instillate post-surgery (study extension), the same magnitude of reduction in incidence was not seen (incidence of adhesion-free sites was 16%, compared with 50% using icodextrin wash plus instillate and 5% for surgical controls). However, whilst the effect of 4% icodextrin wash plus instillate was significantly greater, reduction in the rank of overall scores following 4% icodextrin wash alone was also statistically significant compared with the surgical controls ($P < 0.001$) (Table II). Overall rank scores were less severe in the study extension (mean surgical control score of 25, in contrast to 86 in the main study), although the effects of 4% icodextrin wash plus instillate remained fairly constant (78% reduction in the extension and 68% in the main study). Corresponding reductions of overall rank scores by 4% icodextrin wash only (Table II) and by 4% icodextrin instillate only (Figure 5) were 33 and 68% respectively, both these effects being significant ($P < 0.001$) versus surgical controls within their own study. Moreover, after intra-operative washing only with 4% icodextrin, there was a significant increase in the incidence of adhesion free sites between the bowel and the horns (10/20 versus 2/20; $P = 0.016$) and a significant reduction in the extent of adhesion formation to the bowel ($P = 0.012$) when compared with surgical controls. These data suggest a difference in the formation of non-surgical site adhesions with the use of 4% icodextrin for washing during surgery, in addition to post-operative instillation. However, consistent with the main study, post-operative instillation of icodextrin appears to have the predominant effect overall. No excess fluid or inflammation was associated with the administration of icodextrin.

**Sidewall formation and reformation model**

Administration of 50 ml of 4% icodextrin at the end of the initial surgery in this model resulted in a significant reduction of adhesion formation ($P < 0.001$) in the icodextrin treated group when compared with control. In the icodextrin treated group 10/13 rabbits (77%) had no evidence of adhesions, whilst none of the control animals was free of adhesions (Table III). Control animals from this first part of the study underwent adhesiolysis and nine of the animals had a post-operative instillate of 50 ml 4% icodextrin, serving as the treatment groups for adhesion reformation assessment, the controls ($n = 10$) receiving no instillate post-adhesiolysis. The area of sidewall involved in adhesions at the adhesiolysis stage (reformation baseline, as percentage of initial area, Table III) was determined prior to adhesiolysis, and was compared with the area involved at necropsy, 7 days later. The control groups showed a slight, non-significant, reduction in area involved with adhesions, but all animals still had evidence of adhesions. In contrast, in the group treated with 4% icodextrin post-adhesiolysis, there was a significant decrease in the area involved in adhesions ($P < 0.001$) and four of the nine rabbits were completely adhesion free. No excess fluid or inflammation was observed in the abdominal cavity at necropsy.

**Infection potentiation model**

Administration of 20 ml/kg (4 ml) of RLS or 4% icodextrin did not affect the survival of the rats at either level of bacterial inoculum (Table IV). There was no difference between the treatment groups in the incidence of abscesses, when compared within a level of bacterial inoculum. An increase in the overall abscess score was observed after 4 ml of RLS, and was significant at only one concentration of bacterial inoculum ($LD_{50}$, $P \leq 0.05$). No increase in effect was observed after administration of 4 ml 4% icodextrin at either level of bacterial inoculum when compared with surgical control. No excess fluid or inflammation was observed in the abdomen at necropsy.

**Table II. Effect of 4% icodextrin on adhesion formation when used as an intraoperative wash with and without a post-operative instillate (rabbit double uterine horn model: study 3 extension)**

<table>
<thead>
<tr>
<th>Wash</th>
<th>Instillate</th>
<th>% of Horn involved (overall scores)</th>
<th>Rank of overall scores (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Right horn adhesions</td>
<td>Left horn adhesions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bowel</td>
<td>Bladder</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>36 ± 6.0</td>
<td>41 ± 5.9</td>
</tr>
<tr>
<td>4% icodextrin</td>
<td>None</td>
<td>13 ± 5.6</td>
<td>33 ± 4.2</td>
</tr>
<tr>
<td>4% icodextrin</td>
<td>4% icodextrin</td>
<td>6 ± 4.3</td>
<td>13 ± 4.5</td>
</tr>
</tbody>
</table>

***$P < 0.001$ a versus surgical controls, b versus 4% icodextrin wash. R = right; L = left.
Table III. Effect of administration of icodextrin at the end of surgery on adhesion formation and reformation in rabbit sidewall models (% of initial area)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>% Area affected at baseline Mean ± SEM</th>
<th>% Area affected at necropsy Mean ± SEM</th>
<th>Number adhesion free</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control formation</td>
<td>19a</td>
<td>–</td>
<td>92.5 ± 5.9</td>
<td>0a</td>
</tr>
<tr>
<td>4% icodextrin formation</td>
<td>13</td>
<td>–</td>
<td>6.7 ± 3.6***</td>
<td>10</td>
</tr>
<tr>
<td>Control reformation</td>
<td>10</td>
<td>93.0 ± 4.0</td>
<td>78.0 ± 7.1</td>
<td>0</td>
</tr>
<tr>
<td>4% icodextrin reformation</td>
<td>9</td>
<td>92.0 ± 5.9</td>
<td>26.7 ± 10.1***</td>
<td>4</td>
</tr>
</tbody>
</table>

***p < 0.001 versus surgical controls.

*a These 19 animals continued to the reformation study, 10 to the control group and nine to the icodextrin treatment group.

Table IV. Effect of administration of 4 ml Ringer’s lactated solution (RLS) or 4% icodextrin on survival, the rank of the overall abscess score, and the incidence of abscess formation in the rat infection potentiation model

<table>
<thead>
<tr>
<th>Group</th>
<th>Survival: number of deaths/total</th>
<th>Rank of overall abscess score Mean ± SEM</th>
<th>Number of sites abscess-free</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD10 Control</td>
<td>1/15</td>
<td>19.9 ± 3.35</td>
<td>18/56 4 sites × 14 rats</td>
</tr>
<tr>
<td>4 ml RLS</td>
<td>1/15</td>
<td>26.5 ± 2.70</td>
<td>21/56</td>
</tr>
<tr>
<td>4 ml 4% icodextrin</td>
<td>1/15</td>
<td>18.2 ± 3.24</td>
<td>21/56</td>
</tr>
<tr>
<td>LD50 Control</td>
<td>6/15</td>
<td>10.6 ± 2.74</td>
<td>6/36 4 sites × 9 rats</td>
</tr>
<tr>
<td>4 ml RLS</td>
<td>6/15</td>
<td>19.5 ± 1.70ab</td>
<td>3/36</td>
</tr>
<tr>
<td>4 ml 4% icodextrin</td>
<td>6/15</td>
<td>11.9 ± 2.45</td>
<td>8/36</td>
</tr>
</tbody>
</table>

*P < 0.05 a versus surgical controls, b versus 4% icodextrin.

Discussion

In order to reduce i.p. adhesion formation and reformation, an agent must separate damaged surfaces during the fundamental phases of post-surgical repair. A substantial body of clinical data is available to assess the benefit of crystalloid instillates, such as saline and RLS, in adhesion prevention. A combination of these studies showed an adhesion reformation rate of ~80% in patients who received crystalloid instillates (Fayez and Schneider, 1987; diZerega and Campeau, 1994). Other reports describe the use of 300–500 ml crystalloid solutions to reduce adhesion formation after laparoscopic ovarian surgery. (Naether and Fisher, 1993). When compared to a previous series treated in a similar fashion by these authors but without the crystalloid instillation, there was no difference in the incidence of adhesions (17 versus 19% respectively). Meta-analysis of clinical studies using crystalloid solutions showed no overall reduction in adhesion formation with instillation of lactated Ringer’s solution or saline (Wiseman et al., 1998). The rapid rate of absorption of crystalloid solution from the peritoneal cavity (35 ml/h) probably precludes its residence during the critical time of adhesion formation (Shear et al., 1965; Hart and Magos, 1996). These clinical findings suggest that post-operative instillation of a crystalloid solution is unlikely to have a marked effect on adhesion formation in the clinical setting. Direct comparison with animal studies is inadvisable because of the relatively small volumes generally used in clinical studies (200–300 ml, being about 5 ml/kg at most).

However, we have found little effect in animals when using 20 ml/kg instillates of saline and RLS.

In a rat model of adhesion formation, peritoneal dialysis (PD) was performed for 24 h post-operatively in an attempt to reduce adhesions, using an electrolyte solution (Lindenberg, 1982). This process reduced adhesion formation, presumably due to a flotation effect, disruption of early-stage adhesions and/or dilution of fibrin exudate. Although PD is not feasible in the clinical setting post-operatively, the result stimulated further investigations. In another study in rats (Bhata and Allen, 1997), a reduction in adhesion formation was observed using a hyperosmolar PD solution, compared with either saline or surgical controls. In-vitro studies (Sitter et al., 1999) have demonstrated pro-fibrinolytic stimulation of the synthesis of tissue plasminogen activator (t-PA) in human peritoneal mesothelial cells by hyperosmolar glucose PD solutions. This was mimicked by metabolically inert hyperosmolar isomers, whereas the iso-osmolar icodextrin 7.5% PD solution had no effect. In clinical use, it was suggested, co-stimulation of fibrinolysis by hyperosmolar glucose PD solutions may help to balance glucose-induced extracellular matrix deposition (Sitter et al., 1999).

Icodextrin 7.5% (Extraneal™; Baxter Healthcare Inc.) solution is licensed for use in PD; although iso-osmolar, it induces ultrafiltration through colloid osmosis. Compared with hyperosmolar dextrose-based PD solutions, icodextrin appears to have fewer adverse effects on the mesothelial cells lining...
Icodextrin pre-clinical adhesion prevention studies

the peritoneal cavity (Ho-dac-Pannekeet et al., 1996), and on the defence function of human peritoneal cells (Liberek et al., 1993; Topley et al., 1994; Thomas et al., 1997). There are now >4000 patient years of experience with the 7.5% icodextrin solution in patients with renal failure. The i.p. residence time for a 4% icodextrin solution in humans has recently been demonstrated to be at least 72–96 h, in comparison with saline and dextrose-based PD solutions which are fully resorbed before 24 h (Gilbert et al., 1999). The studies described in this paper show that a new solution based on the glucose polymer, icodextrin, is significantly more effective than crystalloid solutions in animal models of PSA formation.

Apart from crystalloid instillates, site-specific agents have shown clinical efficacy at reducing PSA formation [Interceed (TC7) Adhesion Barrier Study Group, 1989; Azziz, 1993; Diamond, 1996]. These, however, require the surgeon to predict likely sites of adhesion formation, can be awkward to use, particularly during laparoscopies, and do not protect against non-surgical site adhesions. Our preclinical studies have shown the new icodextrin solution to be significantly effective in reducing both site and non-site adhesions in animal models of PSA formation.

We have demonstrated that icodextrin is effective in at least two models of PSA formation (rabbit DUH and sidewall), reflecting its potential for use in both gynaecological and general abdominal surgery. The volume of 20 ml/kg icodextrin was chosen for these experiments because smaller volumes were not as effective and volumes above this showed no advantage. Similarly, a lower concentration of icodextrin than 4% was less successful in preventing PSA formation, with no advantage accruing from the use of higher concentrations. Maximal efficacy (with no effect on the course of bacterial peritonitis) was observed after administration of 20 ml/kg of 4% icodextrin used both intra-operatively and as a post-operative instillate. Although a highly significant benefit was seen using 4% icodextrin as a post-operative instillate alone (whether compared with surgical controls, saline or Ringer’s lactated solution), trends in the incidence of adhesion-free non-surgical sites in the DUH model are suggestive of an additive effect, showing an increase of 12% with 4% icodextrin wash plus instillate over instillate alone. This trend is supported by the separate demonstration of a significant improvement in the incidence of adhesion-free sites (compared with surgical controls), after intra-operative washing alone with 4% icodextrin, plus a further significant improvement when followed by post-operative installation of 4% icodextrin. Differences in the severity of surgical control values preclude direct comparisons between these studies, and firm conclusions regarding the possible additive effects of icodextrin wash and instillate in the DUH model would require a further, specific study. However, the less obvious benefits of intra-operative washing with 4% icodextrin may remain masked by the consistently predominant effect of post-surgical instillation.

A 50 ml instillate of 4% icodextrin also reduced adhesion formation significantly in the rabbit sidewall model. The reduction in PSA formation demonstrated in these preclinical studies encompassed not only the incidence, but also the extent and severity of adhesion formation. Furthermore, 4% icodextrin solution has been shown to have no effect on the exacerbation of the course of bacterial peritonitis. These data suggest that 4% icodextrin should be evaluated as a potential agent for the reduction of PSA formation in the clinical setting, and such studies are now ongoing.

Acknowledgements

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