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Oxychlorine species suppress postsurgical adhesions in rats

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ARTICLE INFO

Article history:
Received 5 May 2013
Received in revised form
24 June 2013
Accepted 23 July 2013
Available online xxx

Keywords:
Surgical adhesions
Chlorine dioxide
Free radical scavenger
Peritonitis
Antisepsis

ABSTRACT

Background: Surgically induced adhesions complicate up to 100% of abdominal surgeries. Food and Drug Administration–approved treatments are generally not only less effective than desired but they also have major contraindications. Oxychlorine species, including chlorine dioxide (ClO_2), suppress scar formation in infected wounds without affecting keratinocytes while reducing fibroblast proliferation. The aim of the present study was to evaluate the effect of oxychlorine solutions containing ClO_2 on adhesion formation.

Methods: Male Wistar rats were subjected to Buckenmaier model of surgical adhesions and treated with either oxychlorine solutions containing ClO_2 (40–150 ppm) or isotonic saline solution. To increase the severity of adhesions, peritonitis was produced by intraperitoneal administration of a diluted nonlethal dose of feces (50 mg/kg). Wound strength of the healed wound was measured to evaluate the effects of oxychlorine solutions. In addition, an oxychlorine solution of lesser efficacy (at 100 ppm) was compared with three available anti-adhesion materials.

Results: Reproducibility of the model was validated in 26 rats. Oxychlorine solutions containing ClO_2 (40–110 ppm) significantly reduced postsurgical adhesion formation without affecting the strength of the healed wound. Higher concentrations (120 and 150 ppm) had no effect. Fecal peritonitis significantly increased, and solutions with ClO_2 at 110 ppm significantly reduced adhesion formation. The effect of the oxychlorine solution was significantly greater than that of Interceed, Guardix, Seprafilm, and isotonic saline solution.

Conclusions: ClO_2-containing oxychlorine solutions could be an innovative strategy for the suppression of surgical adhesion formation, with the additional advantage of contributing antiseptic properties.

1. Introduction

An adhesion is a fibrous bridge between two or more tissues that blocks their normal free movement [1]. The main cause of adhesions is a surgical procedure (surgical adhesions) of almost any type. Up to 100% of patients submitted to abdominal surgery will develop adhesions [2]. The clinical symptoms of abdominal adhesions range from nothing (asymptomatic) to chronic abdominal pain, female infertility and bowel obstruction [1–3]. Bowel obstruction produced by adhesions

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0022-4804/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2013.07.043

frequently requires subsequent surgery to sever the adhesions (adhesiolysis) and liberate the bowel [3]. Although it is known that as many as 53% of patients submitted to adhesiolysis will have a recurrence, there is no way to predict who will develop adhesions after their first abdominal surgery [4]. The 1994 National Hospital Discharge Survey estimated that in the United States, adhesiolysis procedures cost $1.3 billion annually (hospitalization and surgical expenditures) [5], which clearly underscores the need for an effective treatment that could reduce or even potentially eliminate postsurgical adhesions.

Different oxychlorine species have been used extensively as disinfectants [6]. Hypochlorite–hypochlorous acid (bleach) is the most well-known example. Chlorine dioxide (ClO_2), another oxychlorine species which has come into prominence more recently, is a stable, free radical molecule capable of oxidizing organic and inorganic species [6]. Apparently, the disinfectant effect of ClO_2 is attributable to the oxidation of certain microbial biomolecules containing S–H and/or S–S groups (in certain sulfur amino acids), the amino acids phenylalanine and tryptophan, and other labile species. Tetrachlorodecaoxide complex, a chlorite–peroxide oxychlorine species, first used as an antimicrobial agent for wound infections was also found to affect wound repair in humans [7]. In an investigation for preventing surgical adhesion formation in rabbits [8], the authors found that tetrachlorodecaoxide decreased adhesions by 25% compared with saline solution controls [8]. Kenyon *et al.* [9] studied the effect of a composition named "Alcide" (an acidified chlorite system: a mixture of oxychlorine species) on wound repair in guinea pigs. They found that *Pseudomonas*-infected wounds treated with Alcide as an antiseptic gel after healing had formed less collagen and less scarring than isotonic saline solution (ISS) controls, although both had the same epithelialization. The authors postulated that Alcide decreased fibroblast stimulation by activated macrophages.

The aim of this study was to determine whether certain proprietary ClO_2-containing oxychlorine solutions can prevent abdominal surgical adhesion formation in an experimental animal model, even in the presence of contaminating microorganisms, such as associated with nonlethal fecal peritonitis. Additionally, the study compared the new technology with three available anti-adhesion compositions.

2. Methods

2.1. Animals

All the experiments were performed according to the Institutional Ethical Committee for Animal Research (Comite para la Investigacion, Cuidado y Uso de Animales de Laboratorio [CICUAL]). Male Wistar rats (230–250 g, 10-wk old) were used in the experiments. The animals were kept at the Institutional Animal House, fed with Purina Chow (Purina, Mexico) and water *ad libitum*, and subjected to light–dark cycles of 12 × 12 h.

2.2. Surgical procedure

Surgical adhesions were produced following the model reported by Buckenmaier [10]. We chose this model based on a publication comparing four different techniques to produce abdominal surgical adhesions in the rat, and the authors concluded that Buckenmaier model was the most consistent and reproducible [11]. In addition, analyzing the variability in a group of 26 rats validated reproducibility (see Results section). In brief, the animals were anesthetized with sodium pentobarbital (45 mg/kg, intramuscularly), the abdomen was shaved and disinfected with povidone iodine, a middle incision was made, and the parietal peritoneum wall was then exposed. Four vessels were located on the left side of the parietal peritoneum (the distance between the vessels was at least 1 cm) and then ligated with 3-0 silk suture to produce ischemia. The peritoneum around the suture was gently abraded with the tip of scissors, and the incision was then closed in two layers. The peritoneum and abdominal muscles were closed with continuous 3-0 prolene suture, and the skin was closed with continuous 3-0 Dermalon (Kendall Co) suture. Before the peritoneum was completely closed, 10 mL of control or treatment solutions was infused into the peritoneal cavity: either a sterile ISS (control) or a sterile oxychlorine saline (40–150 ppm ClO_2).

2.3. Evaluation of adhesions

Seven days after surgery, the animals were sacrificed by overdoses of sodium pentobarbital (200 mg/kg, intraperitoneally); the abdominal cavity was opened carefully, photographed, and the adhesions then counted (and confirmed in the photographs). For each rat, an adhesion score was recorded as follows: 1 point was assigned to each adhesion formed on the ischemic buttons (Buckenmaier model) and additionally 2 points assigned to any of the following cases: adhesions formed between portions of the intestines, adhesions to the liver, or adhesions to the peritoneal wound. In this way, the maximum adhesion score per rat would be 10 (1 point per each of the four ischemic buttons plus 2 points for adhesions between the intestines plus 2 points for adhesions to the liver plus 2 points for adhesions to the peritoneal wound).

2.4. Contamination of the peritoneal cavity

Because contamination of the peritoneal cavity is known to aggravate adhesion formation [12], a modification to Buckenmaier model was carried out. One hour before surgery (see 2.2. Surgical procedure), 44 rats were treated intraperitoneally with a solution made with their own feces (50 mg/kg, diluted in 400 μL of ISS). Previous experiments were run, at decreasing concentrations, until a dose was established that did not kill the animal but did induce significant peritonitis (nonlethal fecal peritonitis). The animals were divided into four groups (at least seven animals per group): one received ISS (after the surgical and treatment procedures mentioned above) and the other three were treated with the oxychlorine solution containing ClO_2 at 40, 70, or 110 ppm. Sacrifice and evaluation of the adhesions were done as described previously.

2.5. Measurement of wound strength

One of the concerns using a treatment that could affect fibroblast growth (to reduce adhesion formation) is that it

could adversely affect normal healing of surgical wounds. After sacrifice and before opening the abdominal cavity of the fecally contaminated animals, wound strengths were measured according to the method reported by Marques Batista *et al*. [13]. In brief, a surgical glove finger, attached to a mercury sphygmomanometer through a 16-F Foley catheter, was introduced into the peritoneal cavity through a 0.5-cm incision of the skin and peritoneal wounds. The incision was carefully closed around the Foley catheter and the glove finger inflated until the surgical wound was forced open and the finger emerged. The pressure needed to open the wounds was recorded in millimeters of mercury.

2.6. *Comparative study*

To have a more clinically useful evaluation, an oxychlorine solution was compared (n at least 6 per group) with three available anti-adhesion compositions: Guardix (Hanmi Medicare Inc, hyaluronic acid and sodium carboxymethyl cellulose gel) [14], Interceed (Johnson and Johnson Co, oxidized regenerated cellulose) [15], and Seprafilm (Genzyme Corp, hyaluronic acid and carboxymethyl cellulose barrier) [2,15]. An additional control group (ISS) was used in this series of experiments. After surgery (see above), the animals received 10 mL of ISS (control group), 10 mL of an oxychlorine solution (containing ClO_2 at 100 ppm), Guardix (400 μL per rat), Interceed (1.5 cm^2 applied on each ischemic button), or Seprafilm (1.5 cm^2 applied on each ischemic button). One hundred μL of ISS was applied on each Interceed or Seprafilm square after they were placed on each ischemic button. The experiment was finished and the evaluation of adhesion scores was performed as mentioned previously.

2.7. *Experimental groups*

1. Seven groups (at least $n = 6$ per group): Treated with ISS (control) or oxychlorine solutions containing 40, 70, 96, 110, 120, or 150 ppm of ClO_2.
2. Four groups with abdominal cavity contamination: Control ($n = 21$, ISS) and three groups treated with oxychlorine solutions containing ClO_2 at 40, 70, or 110 ppm ($n = 9$, 7, and 8, respectively).
3. Five groups were used for the comparative study: Control ($n = 8$, ISS), Interceed ($n = 8$), Guardix ($n = 8$), Seprafilm ($n = 6$), and an oxychlorine solution containing ClO_2 at 100 ppm ($n = 9$).

2.8. *Preparation of the proprietary ClO_2-containing oxychlorine solutions*

The proprietary solution (Kross-Link Laboratories, Bellmore, NY) [16] is prepared in a sterile bag of ISS by sequential addition of sodium chlorite, a solution of sodium hypochlorite combined with sodium carbonate, and then a citric solution. This acidity converts hypochlorite ion to hypochlorous acid and oxidizes part of the chlorite ion to chlorine dioxide. At the same time, the combination of citric acid and the alkalinity from the hypochlorite forms a citrate buffer. We therefore have two physiological buffers present in the saline solution mixture that now contains the desired amount of chlorine dioxide and unconsumed chlorite ion. This is accomplished by judicious selection of the levels of chlorite, hypochlorite, and citric acid, to create our oxychlorine system, consisting of chlorine dioxide and chlorite ion. The two species form a particularly effective oxidation pair, the $[Cl_2O_4]^-$ ion. The level of ClO_2 used to characterize the specific oxychlorine solution prepared was measured by its ultraviolet absorption at 360 nm, using an extinction coefficient of 1243 L/M cm.

2.9. *Statistical analysis*

All values are presented as the mean ± standard error of the mean. The adhesion index was evaluated using the Kruskal–Wallis test (Dunn multiple comparison test *post hoc*) or Mann–Whitney test; wound strength was evaluated using the unpaired t-test. Differences were considered significant if P was <0.05. All the statistical analyses were performed using Prism 5.0b (GraphPad Software Inc, San Diego, CA).

3. Results

The reproducibility of the model was demonstrated with 26 rats treated with ISS. The adhesion score was 4.0 ± 0.2 (ISS, Fig. 1).

A dose–response curve was performed for oxychlorine solutions containing ClO_2 from 40–150 ppm. Concentrations from 40–110 ppm significantly reduced adhesion formation (Figs. 1 and 2A and B), whereas oxychlorine solutions containing ClO_2 at 120 or 150 ppm did not change the adhesion score (compared with control).

Adhesions significantly increased in control animals (treated with ISS) with fecal peritonitis (adhesion score of 4.0 ± 0.3 without fecal peritonitis and 5.6 ± 0.3 with fecal

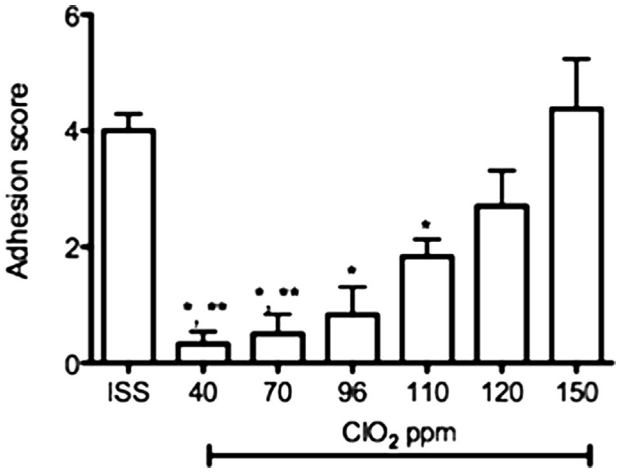

Fig. 1 – Dose–response curve of oxychlorine solutions. Reproducibility of Buckenmaier model is evident in the control group (ISS, $n = 26$). The oxychlorine solutions (ClO_2 concentration from 40–110 ppm, n at least 6 per concentration) significantly prevented adhesion formation ($P < 0.0001$, Kruskal–Wallis test; $^*P < 0.05$ compared with ISS, $^{**}P < 0.05$ compared with 150 ppm, Dunn multiple comparison test), whereas ClO_2 concentrations of 120 and 150 ppm had no effect ($P > 0.05$ compared with ISS). Data are shown as the mean ± standard error of the mean.

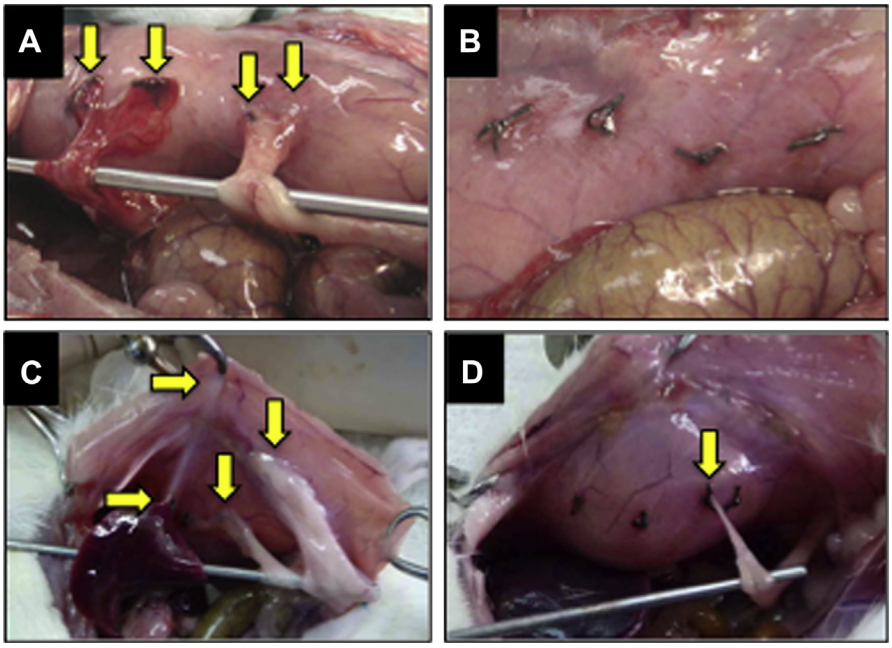

Fig. 2 – Representative photographs showing the effect of oxychlorine solutions on adhesion formation. The four animals were subjected to Buckenmaier model. (A) and (C) are from control animals (treated with ISS), whereas (B) and (D) were treated with an oxychlorine solution containing ClO_2 at 110 ppm. (A) and (B) are without fecal contamination, whereas (C) and (D) are with fecal contamination. The arrows show adhesions. There are four adhesions (to ischemic buttons) in (A); (B) shows no adhesions; (C) shows adhesions to the ischemic buttons, the liver, and the peritoneal wound (the later is a thick adhesion); and (D) shows one thin adhesion to one of the ischemic buttons.

peritonitis; $P = 0.003$). Even in the presence of fecal peritonitis, the oxychlorine solution (containing ClO_2 at 110 ppm) significantly reduced adhesions (Figs. 2C and D and 3A). Oxychlorine solutions containing ClO_2 at 40 or 70 ppm did not change the adhesion score obtained in the control group with contaminated peritoneal cavities (Fig. 3A). Treatment with the oxychlorine solution (containing ClO_2 at 110 ppm) did not change the wound strength (Fig. 3B).

The results of the comparative study (Fig. 4) showed no difference between ISS, Interceed, Guardix, and Seprafilm, whereas the oxychlorine solution significantly reduced the adhesion score.

4. Discussion

The main finding of the study was the capacity of ClO_2-containing oxychlorine (40–110 ppm) solutions to suppress and even prevent the development of surgically induced adhesions. The limitation of the study was the experimental model requirement for permanent silk sutures in the peritoneal cavity, which is not a condition in the clinical field. However, the model has been shown to be reproducible, and it was chosen based on a publication analyzing different models of surgical adhesions in which the authors concluded that Buckenmaier model was the most consistent and reproducible [11].

Certain factors have been identified in the past as triggers of adhesion formation. Such factors include foreign material (e.g., starch, surgical gloves, certain sutures), infection, and duration of surgery [2]. Even when these factors are carefully managed, adhesions are still formed. It now appears reasonable to suggest that the process of adhesion formation begins at the very moment of the surgical incision, when tissues are exposed to environments to which they had never been exposed. Additionally or alternatively, the incision interrupts blood circulation, leading to the production of hypoxia. Indeed, postsurgical tissue attachments have been demonstrated as early as 2 h after surgery in a rodent model [17], which suggests that the actual process of adhesion formation begins earlier. It has been postulated that hypoxia plays an important role in adhesion formation [18]. In fact, when normal fibroblasts are subjected to hypoxia, their phenotype is changed, resembling adhesion fibroblasts [19].

It is known that hypoxia triggers superoxide production and inflammation [20]. Superoxide acts as a signal transduction factor inducing inflammation [21]. Chlorine dioxide is a paramagnetic stable-free radical with powerful oxidant properties [22,23]. Our hypothesis is that oxychlorine species, including ClO_2, function by stabilizing superoxide and decreasing the inflammatory cascade triggered by hypoxia. Indeed, it was previously demonstrated, by electron paramagnetic spectroscopy, that an oxychlorine solution containing 15 ppm of ClO_2 scavenged 100% of superoxide produced by xanthine and xanthine oxidase (an effect comparable with that produced by superoxide dismutase and catalase) [24]. The half-life of ClO_2 is very brief (within minutes), in the presence of oxidizable organic matter, suggesting that the effect is produced very early in the process of adhesion formation, thereby leaving the subsequent healing process intact. Indeed, the wound strength was not affected by the oxychlorine solution.

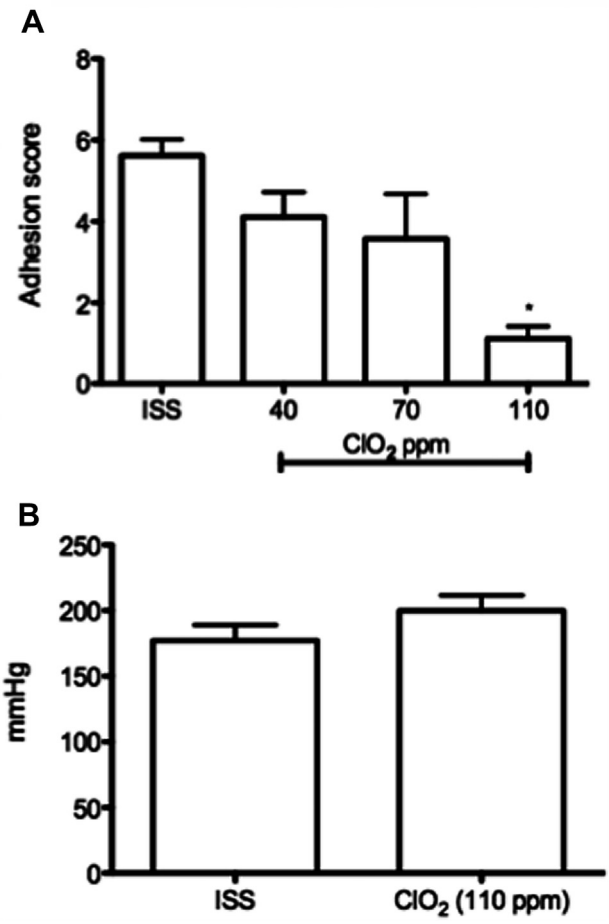

Fig. 3 – Effect of oxychlorine solutions on adhesion formation and wound strength. All the animals were subjected to nonlethal fecal peritonitis. The oxychlorine solution (ClO_2 at 110 ppm, $n = 8$) significantly reduced adhesion formation in (A) ($^*P = 0.0003$, Mann–Whitney test, compared with ISS, $n = 7$), whereas it did not change the wound strength in (B) ($P = 0.19$, unpaired t-test). The oxychlorine solutions containing ClO_2 at 40 and 70 ppm had no effect ($P > 0.05$, A). Data are shown as the mean ± standard error of the mean.

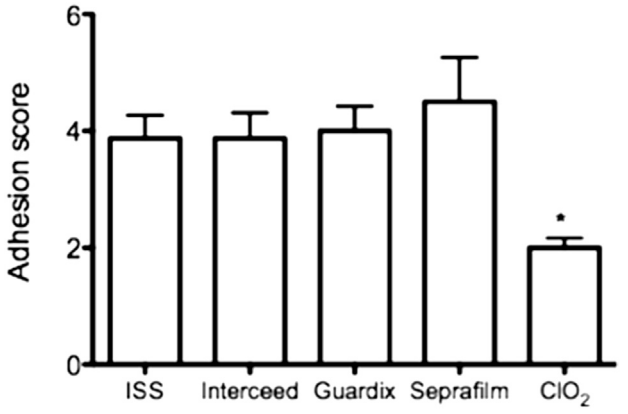

Fig. 4 – Comparative study. Interceed, Guardix, and Seprafilm had the same effect as ISS. The oxychlorine solution containing ClO_2 at 100 ppm significantly reduced adhesion formation ($P = 0.003$, Kruskal–Wallis test; $^*P < 0.05$ compared with ISS, Dunn multiple comparison test). Data are shown as the mean ± standard error of the mean.

Intriguingly, we observed an inverse dose–response curve for the oxychlorine solutions depending on ClO_2 concentration. The higher the concentration the smaller is the effect. It should be noted, however, that these results were obtained in the absence of peritoneal contamination, wherein the study using an oxychlorine composition containing 100 ppm of ClO_2 showed superior efficacy compared with the three commercial products. The fecal (organic) contaminants significantly depleted the efficacy of the lower level ClO_2-containing oxychlorine solutions. It is reasonable to conclude, based on our experience with these complex oxychlorine systems, that two competing actions are at play. In the absence of significant organic matter, the full oxidative capacity of ClO_2–chlorite systems can be directed fully to stabilize superoxide and decrease the inflammatory cascade triggered by hypoxia. When competing oxidizable targets, such as manifest by fecal contaminants, are present, the lower levels of 40 and 70 ppm of ClO_2 in the treatment solutions are being partially vitiated by those targets. Only at 100 ppm level, there is sufficient remaining activity available to quash the superoxide and suppress the hypoxia-triggered inflammatory cascade. In contrast, when considering the decrease in adhesion suppression, where the score for the 40 ppm system shows remarkable activity compared with the proportionately diminishing suppression at higher levels of ClO_2-containing oxychlorines, it is a reasonable conclusion by these authors that the known cytogenic effects of excess chlorite ions are increasingly coming into play. The higher the chlorite level, above what is needed to function as component of the $[Cl_2O_4]^-$ ion, the greater is the competitive adverse effect on adhesion suppression. Clearly, the choice of treatment solution to be used in a surgical intervention must reflect the condition of the subject. These authors are now undertaking the obvious next phase of the studies, whereby the relative ratio of oxychlorine components [ClO_2:chlorite ion] will be optimized for the intended surgery. In the bulk of surgical interventions, *sans* organic contaminants (e.g., bacterial infections), the choice will be easier. Future studies will be directed to provide more detailed understanding of these complex but obviously highly effective oxychlorine systems.

The Food and Drug Administration has approved at least three synthetic products that presumably function by separating the surgically injured tissue from other tissue surfaces during surgery [2]. However, these products are often poorly effective and actually contraindicated if there is infection or bleeding in the surgical subjects. Oxychlorine solutions, which are powerful germicides [24], in contrast, could be favorably used in these circumstances. In fact, oxychlorine solutions were demonstrably effective even in the presence of fecal peritonitis. This could represent a major advance in the treatment of surgical adhesions.

It is therefore concluded that oxychlorine solutions prevented surgical adhesion formation in the experimental model and can be an innovative strategy for the suppression of adhesion development in surgical subjects, with the additional advantage of the system's inherent antiseptic properties.

Acknowledgment

This work was funded by grant SIP20121039 from the Instituto Politecnico Nacional. The authors are grateful to Iriabeth Guzman, whose help in the experiments was important in their accomplishment.

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