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Effects of Contractubex on the Prevention of Postoperative Peritoneal Adhesion

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Background. **There are numerus techniques and agents in use for reducing peritoneal adhesion formation. But in this research we believe this is the first research to reveal that contractubex (allium cepae, sodium heparin, and allantoin mixture) is reducing formed peritoneal adhesions. So it may be used to reduce the number of re-laparotomies/re-laparoscopies caused by peritoneal adhesions related complications.**

Objective. **To evaluate the effects of contractubex (CT) in a rat model of postoperative peritoneal adhesion (PPA).**

Methods. **Fifty rats were divided into four equal groups. In group 1, 1 g of CT was injected into the peritoneal cavity. In group 2, adhesions were generated. In group 3, adhesions were generated, and 1 g of CT was immediately applied into the peritoneal cavity. In group 4, adhesions were generated, and at postoperative d 7, 1 g of CT was applied into the peritoneal cavity. In group 5, adhesions were generated, and at postoperative d1, 3, 5, and 7, 1 g of CT was applied into the peritoneal cavity. The adhesions were scored both macroscopically and microscopically.**

Results. **The mean macroscopic adhesion scores in groups 1–5 were 0 ± 0, 2.9 ± 0.21, 2.3 ± 0.54, 0.8 ± 0.63, and 2.2 ± 0.72, respectively ($P < 0.0001$); the mean microscopic values were 0 ± 0, 2.8 ± 0.42, 2.8 ± 0.42, 0.6 ± 0.52, and 2.3 ± 0.48, respectively ($P < 0.0001$). The mean macroscopic adhesion score in group 4 was lower than that in group 2 ($P = 0.001$). The mean macroscopic adhesion scores in groups 3 and 5 were higher than those in group 4 ($P = 0.045$ and $P = 0.038$, respectively) but did not significantly differ from those in group 2 ($P = 0.171$ and $P = 0.124$, respectively).**

Commentary. **A single dose of contractubex did not prevent PPA formation but did diminish the amount of formed PPAs.**

Key Words: **contractubex; adhesion; periton; prevention; postoperative.**

INTRODUCTION

Postoperative peritoneal adhesion (PPA) formation is a serious problem in surgery. Among the surgical methods tested in the prevention of PPAs are minimally invasive and laparoscopic techniques. In addition, pharmacologic agents that target fibrin formation, and liquids, gels, and solids that constitute a mechanical barrier between the mesothelial surfaces, have been assessed. Although some of these methods have shown benefits, the complete prevention of PPAs has not been achieved [1–3].

Recent studies have focused on the formation of a mechanical barrier between peritoneal surfaces. Among the materials tested are nonabsorbable solid materials (e.g., amniotic membranes), solids that become absorbable by liquefying after a period of time (e.g., hyaluronic acid), and liquids (e.g., methylene blue) [4–6]. All of these materials are easily applied, relatively inexpensive, and suitable for peritoneal physiology [7].

Of these mechanical barriers, gelatinous liquids with high viscosities have been found to be more beneficial. Highly viscous liquids may prevent PPA formation by forming a layer between surfaces, thereby preventing any contact of the deperitonized surfaces with the surrounding tissues [8, 9]. Similarly, *in vitro* studies showed that in a highly viscous medium, the movement of cells or cell groups towards each other is delayed, and adhesion either does not occur or is delayed, depending on the viscosity [10].

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Contractubex (Merz Pharma, Frankfurt, Germany) is a gel containing primarily allium cepae (onion extract) as well as 50 U sodium heparin and 1% allantoin. In clinical practice, this drug is generally used for hypertrophic scars and keloids. The mechanism of action of the drug is related to blocking the synthesis of connective tissue (i.e., collagens and proteoglicans) [11]. Because the root structure of PPAs is connective tissue, this research aimed to evalute the effects of contractubex on PPAs, a purpose for which this drug has not yet been investigated.

METHODS

This study was performed at the Experimental Animal Production and Research Laboratory of Cerrahpasa Medical School, Istanbul University, and was approved by the local Animal Ethics Committee. All protocols were in accordance with the regulations governing the care and use of laboratory animals of the Declaration of Helsinki.

We planned to utilize five groups of rats, with a sample size power of 0.9 and a 95% confidence interval, which resulted in a distribution of 10 rats in each group. Fifty Wistar out-bred female albino rats (mean weight, 250 ± 25 g; mean age, 5.5 mo) were kept at a maximum of five per cage, in standard 40 × 25 × 25 cm cages with plastic sides and bottoms covered with stainless steel woven wire. The floor of each cage was always covered with wood shavings, which were replaced every 2 d. Rats were fed with pellet feed manufactured specifically for small animals. A 12-h light/12-h dark cycle was used for the illumination of the room where the rats were placed. Approximately 10–12 h before each operation, the feeding boxes were removed to assure gastric emptying prior to surgery.

Anesthesia Technique

Each rat was anesthetized with 75 mg/kg body weight intramuscular ketamine (Ketalar, Parke Davis and Co. Inc., Detroit, MI, USA).

Adhesion Model

The peritoneal cavity was entered through a 3 cm midline incision. The terminal ileum and cecum of all animals were mobilized and placed onto the left index finger of the surgeon. The anterior surface of each cecum was scraped with dry gauze to induce serosal petechiae (scraping model) [12].

Each rat in group 1was intraperitoneally injected with 1 g of contractubex (Merz Pharma), using a 22 French syringe, through a point over the midline of the abdomen and 3 cm below the xiphoid. Each rat in group 2 was placed on its back on an operating table, and its extremities were fixed to the table using sticking plaster. After antisepsis with povidone iodine (Betadine; Kurtsan Co., Istanbul, Turkey), a 3-cm long vertical median incision was made. The cecum was pulled out of the abdomen, and adhesions were induced. The cecum was replaced in the abdomen, and the incision was closed with 3/0 polypropylene sutures (Prolene; Bicakcilar Co., Istanbul, Turkey) using a continuous suture technique.

The rats in groups 3, 4, and 5 were treated in a manner identical to those in group 2. In group 3, 1 g of CT was applied into the peritoneal cavity immediately after adhesions were generated. These rats were sacrificed on d 10. In group 4, 1 g of CT was applied into the peritoneal cavity on d 7, and these rats were sacrificed on d 17. In group 5, 1 g of CT was applied into peritoneal cavity d 1, 3, 5, and 7, and these rats were sacrificed on d 17.

All sacrifices were performed by intraperitoneal injection of 200 mg/kg sodium pentothal. After the rats were sacrificed, the performed laparotomies consisted of reverse U incisions, and any adhesions observed in the peritoneal cavity were graded according to size and severity. Other detected intraperitoneal pathologies were also recorded. For histopatholog evaluation, the adhesion model area of each cecum was resected and preserved in a glass bottle containing formol.

Morphologic Evaluation

Each resected adhesion model area was fixed in formol. After dehydration, the adhesion areas were embedded in paraffin. Cross-sections of 5 μm were prepared, stained with hematoxylin and eosin, and evaluated by light microscopy at a magnification of ×100. All evaluations were performed by a pathologist who was blinded to the methods and groups.

The primary outcome measure was the macroscopic adhesion score, defined as the sum of adhesion severity and adhesion size grading (Table 1). The secondary outcome measure was the histopathologic fibrosis grading of the tissue samples extracted from the adhesion model area (Table 2).

Statistical Evaluation

Statistical analyses were performed using the NCSS 2007 pocket program. The results were evaluated with a confidence interval of 95% and a significance level of $P < 0.05$. In addition to the descriptive statistical methods (mean, standard deviation, median), Kruskal Wallis (KW) test for inter-group comparisons, Dunn's multiple comparison test for the comparison of subgroups, and the χ^2 and Fisher's exact tests for comparison of nonparametric variables were used for the evaluation of the data.

RESULTS

We observed statistically significant differences among several groups (Table 3) in the macroscopic size and severity of adhesions ($P = 0.0001$). The macroscopic adhesion scores of the groups were obtained by calculating the arithmetic means of adhesion size and severity. The mean macroscopic adhesion score in group 4 was lower than that in group 2 (control group) ($P = 0.001$). The mean macroscopic adhesion scores in groups 3 and 5 were higher than that in group 4 ($P = 0.045$ and $P = 0.038$, respectively) but did not significantly differ from those in group 2 ($P = 0.171$ and $P = 0.124$, respectively; Table 4).

We also observed statistically significant differences among several groups (Table 5) in the microscopic histopathological fibrosis values ($P = 0.0001$). The mean

TABLE 1

Macroscopic Adhesion Scoring Classification

Grades	Adhesion size	Adhesion severity
0	No adhesion	No adhesion
1	Presence of adhesion in 25% of the area	Spontaneously separating adhesion
2	Presence of adhesion in 50% of the area	Separation of adhesion with traction
3	Whole area covered with adhesion	Separation of adhesion with a sharp dissection

TABLE 2

Microscopic Histopathologic Fibrosis Evaluation Classification

Grades	Definition
Grade 0	No fibrosis (no fibroblasts and/or collagen fibers)
Grade 1	Slight fibrosis (few fibroblasts and/or collagen fibers)
Grade 2	Median fibrosis (more fibroblasts and/or collagen fibers)
Grade 3	Severe fibrosis (lots of fibroblasts and/or collagen fibers)

histopathologic fibrosis value in group 4 was significantly lower than that in group 2 ($P = 0.001$).

The mean histopathologic fibrosis values in groups 3 and 5 were higher than those in group 4 ($P = 0.01$ and $P = 0.038$, respectively) but did not significantly differ from those in Group 2 ($P = 0.999$ and $P = 0.169$, respectively; Table 5).

DISCUSSION

PPAs result from damage to the peritoneum. The damaged region contains a fibrin-rich exudate, and the fibrins in this exudate form bands between the surfaces in contact with the damaged region. These fibrin bands, as well as the hyaluronic acid-rich matrix that fills the cavity between the bands, provide a suitable environment for collagen synthesis. Actual adhesions occur after such synthesis [1, 13–15].

Contractubex is generally used in clinical practice for the treatment of hypertrophic scars and celoids [11]. Pathophysiologically hypertrophic scars and celoids are characterized by exaggerated extracellular matrix and collagen deposition, as are PPAs [16–18].

The mechanism of action of the drug is revealing time related differenences [19–21]. Contractubex is reducing inflammation and fibroblast proliferation which are the early stage proccesses of the wound healing. Contractubex is also reducing of connective tissue components (proteoglicans, collagen) which is the late stage proccess of the wound healing [19–21]. Group-3 and 4 are designed for that reason; group-3 results were revealed us the early wound healing process effects of contractubex and group-4 were late stage effects.

A prospective, multicenter, clinical research study (38 centers, 859 patients) revealed that contractubex is more effective than topical steroids in the treatment of hypertrophic scars [22].

Contractubex is basically an onion extract, as it contains only 50U sodium heparin and 1% allantoin. Onion extract is a natural onion vegetable product, and it is generally available in Europe as Ccontractubex and in the USA as mederma. Both of these preparations contain 1% allantoin but only contractubex has heparin. Heparin strengthens the anti-inflammatory effects of onion extract and can enhance collagen restoration [23, 24]. The anti-inflammatory effects of onion extract are hypothesized to be due to cepaenes and its antimicrobial effects to thiosulfinates [25].

In the present study, we assessed the potential toxic effects of contractubex on the intact peritoneal cavity by injecting 1 g of contractubex into the peritoneal cavity of the Group 1 rats. We did not use laparotomy for its application in this group because the trauma of laparotomy incisions may have given rise to adhesions, thus leading to false-positive results. After sacrifice, these rats did not reveal any adhesions, toxic effects, or granuloma formation in the peritoneal cavity.

Various approaches have been used to generate experimental PPAs, including abrasion, local excision of the peritoneum, ischemic injury, placement of a foreign body (e.g., talcum powder) in the peritoneal cavity, thermal injury, and bacterial contamination [26–32]. We utilized an abrasion model because of the ability of this model to mimic the mechanical trauma that can occur after laparotomy. Any manipulations performed by hands or surgical instruments during laparotomy can lead to mechanical trauma, and these are the most frequent causes of PPAs [1].

TABLE 3

Macroscopic Adhesion Scores of the Groups and Their Statistical Analysis with χ^2 Test

		Group 1		Group 2		Group 3		Group 4		Group 5		
	Grades	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	
Adhesion size	0	10	100%	0	0	0	0%	3	30%	0	0%	
	1	0	0%	0	0	2	20%	6	60%	3	30%	
	2	0	0%	0	0	5	50%	1	10%	2	20%	χ^2: 70.1
	3	0	0%	10	100	3	30%	0	0%	5	50%	$P = 0.0001$
Adhesion severity	0	10	100%	0	0	0	0%	3	30%	0	0%	
	1	0	0%	0	0	0	0%	6	60%	1	10%	
	2	0	0%	2	20	5	50%	1	10%	6	60%	χ^2: 72.5
	3	0	0%	8	80	5	50%	0	0%	3	30%	$P = 0.0001$

TABLE 4

Mean and Median Values of Macroscopic Adhesion Scores and their Statistical Analysis with Kruskal Wallis Test

Groups	Mean ± SS	Median
Group 1	0 ± 0	0 (0–0)
Group 2	2.9 ± 0.21	3 (2.75–3)
Group 3	2.3 ± 0.54	2 (2–3)
Group 4	0.8 ± 0.63	1 (0–1)
Group 5	2.2 ± 0.72	2.25 (1.5–3)
KW	39.2	
P	0.0001	

KW = Kruskal Wallis.

Because the primary histopathological construction of PPAs shows collagen and extracellular matrix deposition like hypertrophic scars, we sought to evaluate the effects of contractubex on this pathological tissue. We hypothesized that contractubex might diminish already-formed PPAs and/or prevent PPA formation. In Group 3, we applied contractubex immediately after the adhesion model induction, and we found in this group that the macroscopic adhesion scores and microscopic fibrosis values were not significantly different from Group 2 (control group).

However, in Group 4 we applied contractubex into the peritoneal cavity 7 days after the adhesion model induction, and we found statistically significant reductions in both the macroscopic and microscopic results. Thus, these findings indicate that a single dose of contractubex could not prevent PPA formation but it could diminish formed PPAs. In this situation, we decided to evaluate the results of a multi-dose application of ontractubex.

In the literature there are some important examples of this approach. For example, Halofuginone, a specific inhibitor of collagen type I synthesis, was found to significantly lower the PPAs formed using two types of adhesion induction, cecum scraping and uterine horn scraping [33, 34]. In the cited studies, however, Halofuginone was applied daily, either intraperitoneally or orally.

TABLE 5

Mean and Median Values of Microscopic Histopathologic Fibrosis Values and Their Statistical Analysis with Kruskal Wallis Test

Groups	Mean ± SS	Median
Group 1	0 ± 0	0 (0–0)
Group 2	2.8 ± 0.42	3 (2.5–3)
Group 3	2.8 ± 0.42	3 (2.5–3)
Group 4	0.6 ± 0.52	1 (0–1)
Group 5	2.3 ± 0.48	2 (2–3)
KW	41.6	
P	0.0001	

In Group 5, we applied contractubex into the peritoneal cavity four times with two-day intervals, but the revealed results were not statistically different from the control group. Also in that group, we found granulomas in the peritoneal cavity. Upon microscopic evaluation, we found that they were foreign body reactions containing unabsorbed contractubex debris. Multidose contractubex application did not positively affect PPA formation, and we think that the negative mechanism may be related to the over-dose application.

In this research, we have shown that the administration of a single dose of contractubex into the peritoneal cavity of which adhesions are formed decreases the amount of formed PPAs.

The mechanism of this result may be related to effects of contractubex on connective tissue components [19–21]. It is clear that PPA's are construction from connective tissues; collagen and proteoglicans. When the connective tissues are in the area contractubex is reducing them but if these tissues are not, effects of contractubex is getting inadequate.

Thus, for surgical practice, this could be very important. PPAs occur in more than 75% of all laparotomies, and the most often indications of second laparotomies/laparoscopies, female infertility, and postoperative abdominal and pelvic pain are PPAs [35]. The occurrence of PPAs is also a serious economic problem. For example, a multi-center study including all abdominal surgery clinics in Sweden found that the annual economic loss due only to small bowel obstruction was more than US$6 million [36]. Contractubex could be applied with fine needlees that are radiologically guided (for example, using ultrasonography) into the peritoneal cavity for ileus, infertility, and/or abdominal or pelvic pain when the etiology is PPA. However, before clinical application, it will be necessary to perform some new research on this subject; the approprite dose is not clear for humans, and the multi-dose application may be effective if the doses are re-equivalented. The effects of contractubex on the female sex organs is also not clear; it may be toxic and/or destructive when applied directly on or over adnexa.

In conclusion, contractubex is the first drug shown to diminish already-formed PPAs. When it used different ways -for example percutenusely- into the peritoneal cavity, it may be ensure to reduce the number of re-laparotomies/laparoscopies related to PPAs.

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