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Acellular derivatives of mesenchymal stem cells prevent peritoneal adhesions in an animal model

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ABSTRACT

Background: Peritoneal adhesions are nonanatomical connections that bind organs to the abdominal wall or among them. They arise after peritoneal injury, which triggers an inflammatory response followed by a healing process that leads to fibrotic tissue formation. Mesenchymal stem cells and their secretion products, also referred to as acellular derivatives (ACDs), have anti-inflammatory, fibrinolytic, and antifibrogenic properties. The aim of this study was to determine the effect of intraoperative administration of ACD on the appearance, severity, and progression of peritoneal adhesions, in an animal model.

Materials and methods: Cecal erosions were mechanically induced in adult mice. Before closure, the vehicle, ACD, or Seprafilm was administered. Seven days later, the presence and grade of peritoneal adhesions were assessed macroscopically. One, 3, and 7 d after intervention, molecular and cellular markers of inflammation, fibrinolysis, and fibrogenesis were evaluated both locally and systemically.

Results: ACDs avoided the appearance of clinically relevant peritoneal adhesions. The vehicle had no effect, and Seprafilm reduced them inconsistently. The antiadhesive effect of ACD was associated with an early reduction of proinflammatory cytokine (tumor necrosis factor-alpha and interferon-gamma) secretion and leukocyte (polymorphonuclears, mononuclears, and macrophages) infiltration. High levels of D-dimer, low fibrin deposits, low myofibroblasts infiltration, and less fibrosis were also observed.

Conclusions: ACD administered at the end of abdominal surgeries prevents the formation of peritoneal adhesions due to the modulation of inflammatory, fibrinolytic, and fibrogenic processes.

Introduction

Between 60% and 97% of the patients who underwent abdominal surgeries develop peritoneal adhesions.[1] The main complication of peritoneal adhesions is intestinal obstruction, whose incidence reaches 2% for all surgeries and 4% for laparotomic surgeries. The risk of mortality due to intestinal obstructions is over 10%.[1] Thus, postoperative adhesions represent an important health problem as reported in surveys carried out in the United States of America and Europe.[2]

Peritoneal adhesions are nonanatomical fibrous bands located in the abdominal cavity that join abdominal walls and/or organs.[3] They develop in both the injured zone and

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neighboring areas.[1,3] After a mechanical, chemical, or other type of injury, peritoneal mesothelium cells proliferate and restore the epithelial continuity.[2-4] Unfortunately, in some patients, it also appears as a scar tissue in previously virtual spaces. The main pathophysiological steps associated to the development of these peritoneal adhesions are as follows. The first step is inflammation, where residential mastocytes release histamine and platelet-activating factors that increase vascular permeability. Hence, fibrinogen and fibronectin exuded and lead fibrin deposition into injured and healthy areas of the peritoneum.[2-4] Together, chemotactic and inflammatory factors (interleukin [IL]1, IL6, and tumor necrosis factor [TNF]-alpha) are released.[5-7] Also, polymorphonuclear (PMN) cells and macrophages migrate toward the fibrin matrix, undergo phagocytosis of detritus, secrete proinflammatory cytokines (IL1, IL6, TNF-alpha, and interferon [INF]-gamma), promote the invasion of fibroblast (tumor growth factor beta 1), and trigger neovascularization.[2,3,8] The second step is antifibrinolysis because under hypoxia conditions, fibroblasts secrete antifibrinolytic factors (tissue inhibitor of metalloproteinases and plasminogen activator inhibitor). Thus, the fibrin matrix persists, fibroblasts settle, and vessels start growing in the newly formed tissue.[2-5,8,9] The final step is fibrosis because of fibroblast differentiation into myofibroblasts that express smooth muscle alpha actin and over secrete collagen type I.[2,3,8]

Currently, adhesiolysis (surgical removal) is the only available strategy to treat patients who develop peritoneal adhesions. Unfortunately, its recurrence is near 100%.[2,3] To prevent the formation of postoperative adhesions, surgeons should adhere to the good surgical practices (i.e., use talcum-free gloves and minimize organs manipulation).[2,3,10] Together, they could use mechanical barriers commercialized as "preventive adjuvants". Those devices are difficult to handle, and there is no data supporting significant prevention of intestinal obstructions.[10] At preclinical level, various pharmacological strategies have been tested, that is, nonsteroidal anti-inflammatories, corticoids, anticoagulants, gonadotropin-releasing hormone analogs, and statins.[2,3] None of them have shown a robust antiadhesive effect.

Mesenchymal stem cells, also referred to as multipotent stromal cells (MSCs), are a promising therapeutic tool for degenerative, autoimmune, and neoplastic diseases.[11] Several of their beneficial effects are explained, not by their differentiation potential but by their ability to secrete trophic, immunomodulatory, and antifibrotic factors.[11] MSC secretion products, also referred to as acellular derivatives (ACDs), comprise proteins, messenger RNAs, and micro RNA, soluble or packed in microvesicles or exosomes.[12] The following ACD bioactives might impair the development of peritoneal adhesions: (1) IL10, as it reduces neutrophil infiltration, decreases macrophage secretion of tumor growth factor beta 1, and stimulates fibroblasts to secrete metalloproteinases[13-15]; (2) tissue-type and urokinase-type plasminogen activators, as they transform plasminogen into plasmin that degrades fibrin and extracellular matrix proteins[16]; (3) hepatocyte growth factor, as it reduces fibroblast secretion of tumor growth factor beta 1, collagen type I, and collagen type III and increases the production of metalloproteinases 1, 3, and 13[17]; (4) adrenomedullin, as it reduces fibroblast secretion of extracellular matrix components[18]; (5) metalloproteinases, as these zinc-dependent endopeptidases degrade extracellular matrix proteins.[19,20]

The aim of this work was to determine the effect of intraoperative administration of ACD on the appearance, severity, and progression of peritoneal adhesions, in an animal model. For this, cecal erosions were mechanically induced in adult mice. Before closure, the vehicle, ACD, or Seprafilm was administered. Seven days later, the presence and grade of peritoneal adhesions were assessed macroscopically. One, 3, and 7 d after intervention, molecular and cellular markers of inflammation, fibrinolysis, and fibrogenesis were evaluated both locally and systemically (Fig. 1).

Material and methods

Animals

Nine-wk-old female normoweighed and normoglycemic BKS$^{-/-}$ mice were used. Before abdominal surgery, animals received 5-mg/kg tramadol (Saval, Santiago, Chile) and were sedated with sevoflurane (Baxter, Deerfield, IL). Euthanasia was performed administering 50-mg/kg ketamine (Ilium, Argentina), 5-mg/kg xylazine (Centrovet, Santiago, Chile), and 1-mg/kg acepromazine (Drag Pharma, Santiago, Chile).

During the follow-up period, animals were housed in individual cages; had *ad libitum* access to regular diet and autoclaved water; and were maintained at a constant temperature and 60% relative humidity, with a 12:12 light:dark cycle.

Animal protocol was approved by the Ethics Committee of Facultad de Medicina Clinica Alemana-Universidad del Desarrollo (approval ID: 05/2015_CICUAL).

Peritoneal adhesion induction

The procedure described by Dinarvand *et al.*[21] was adapted with minor modifications. Briefly, mice were sedated, the front zone was shaved and disinfected with iodine (Biosano, Santiago, Chile), and the abdominal cavity was opened. The cecum was exposed, eroded with sterile gauze until opacity and erythema occur, and returned to its place. The cavity was closed in two planes.

ACD production, storage, and quantification

Bone marrow–derived MSCs from three young healthy human donors were cultivated in alpha minimum essential medium (Gibco, Gaithersburg, MD) supplemented with 10% fetal bovine serum (Gibco, Gaithersburg, MD) and 80-μg/mL gentamicin (Sanderson Lab, Santiago, Chile). When 80% confluence was achieved, adherent cells were detached with 0.25% trypsin and 2.65-mM ethylenediaminetetraacetic acid and subcultured. At passage two, culture medium was replaced by alpha minimum essential medium. Twenty-four hours later, ACD was collected by aspiration, aliquoted, and stored at −80°C until use. The total protein content of ACD was quantified using the bicinchoninic acid colorimetric kit (Thermo Scientific Pierce, Rockford, IL).

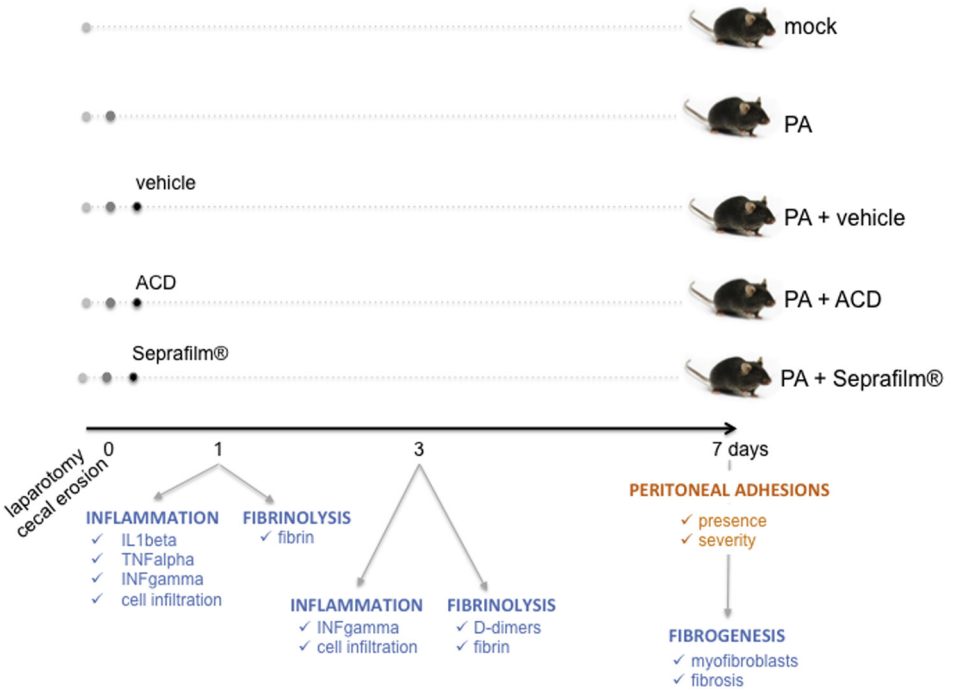

Fig. 1 – Study design. (Color version of figure is available online.)

The vehicle corresponded to alpha minimum essential medium unexposed to MSCs and handled similarly to ACDs.

Vehicle or ACD administration

Just before closing the planes, 0.5 mL of room temperature vehicle or ACD were administered directly into the abdominal cavity.

Seprafilm administration

Mechanical barrier product Seprafilm (Sanofi-Aventis, Bridgewater, NJ) was used as comparator.[1,3,4,10] Just before closing the planes, a rectangle of 1 × 1.5 cm^2 was cut and placed on top of the cecum following manufacturer's instructions.

Macroscopical assessment

Seven days after abdominal surgery, necropsy was performed, opening abdominal cavity through a right flank incision. The absence/presence of peritoneal adhesions was recorded, and their severity was graded according to the scale proposed by Blauer and Collins.[22] Score 0: no adhesive bands; score 1: adhesive band thin and easily removable; score 2: adhesive band thick and limited to one area; score 3: adhesive band thick and extensive; and score 4: adhesive band thick and extensive, between abdominal wall and/or viscera (Fig. S1, Supporting Information).

Local inflammation assessment

Proinflammatory cytokines

One and 3 d after abdominal surgery, 1-mL phosphate buffer saline (Gibco, Gaithersburg, MD) was intraperitoneally injected, and the abdomen was massaged.[23] Peritoneal lavage was collected through the iliac right fossa and centrifuged at 300 × *g* for 5 min. Supernatant was recovered and stored at −80°C.[24,25] IL1 beta, TNF-alpha, and INF-gamma levels were determined using commercial ELISA kits (MyBioSource, Inc, San Diego, CA; R&D Systems Inc, Minneapolis, MN, and eBioscience, Inc, San Diego, CA, respectively).

Infiltrative PMN and mononuclear cells

One and 3 d after abdominal surgery, the cecum was dissected. Fragments of 1 × 0.5 cm^2 including cecum and adhesion tissue (if present) were fixed in formaldehyde and embedded in paraffin. Sections of 5 μm were stained with hematoxylin-eosin (Merck, Kenilworth, NJ). Samples were observed at 400× magnification in an optical microscope (Leica, Wetzlar, Hesse, Germany), and images were captured with a digital camera (Leica, Wetzlar, Hesse, Germany) and analyzed using the ImageJ software (National Institutes of Health, Rockville, MD). PMN and mononuclear cells found outside of the muscular layer of the cecum were counted, and the perimeter of the basement membrane was measured.[26] The infiltration level was expressed as the number of cells divided by the perimeter of the mesothelium. Histological analysis was performed in blind.

Peritoneal macrophages

Three days after abdominal surgery, peritoneal lavage was collected and centrifuged. The pellet was recovered, and cells were resuspended in 1 mL of 0.01% Neutral Red (Sigma–Aldrich, St. Louis, MO) dissolved in phosphate buffer saline. Five minutes later, an aliquot was stained with Trypan Blue (Gibco, Gaithersburg, MD). Cellular counting was performed in a Neubauer chamber. Cells that excluded Trypan

Blue but included Neutral Red were considered as live macrophages.

Systemic inflammation assessment

Proinflammatory cytokines

One and 3 d after abdominal surgery, 1 mL of heparinized blood was collected from the orbital sinus of anesthetized animals. After centrifugation at 2000 × *g* for 20 min, plasma was recovered and stored at −80°C. TNF-alpha and INF-gamma levels were determined using the ELISA kits mentioned previously.

Local fibrinolysis assessment

D-dimer

Three days after abdominal surgery, peritoneal lavage was collected, centrifuged, and stored. D-dimer levels were determined using a commercial ELISA kit (MyBioSource, Inc, San Diego, CA).

Fibrin deposits

One and 3 d after abdominal surgery, histological sections of the cecum were obtained and stained with Masson's trichrome. Samples were observed at 2.5× magnification in an optical microscope; images were captured with a digital camera and analyzed using the ImageJ software. Pericecal fibrin and cecal lumen areas were determined. Pericecal fibrin abundance was expressed as the area occupied by fibrin divided by the area of cecal lumen, times 100.[27] Histological analysis was performed in blind.

Local fibrosis assessment

Myofibroblasts

Seven days after abdominal surgery, histological sections of the cecum were obtained. Tissue sections were deparaffinized, boiled, blocked with 5% fetal bovine serum, and incubated overnight at 4°C with a 1:50 dilution of mouse anti-alpha smooth muscle actin (Cell Marque, Rocklin, CA). Then, samples were washed and incubated 2 h at room temperature with a 1:400 dilution of Alexa 488-conjugated goat antimouse IgG (Cell Signaling, Danvers, MA). Cross-reactivity of the secondary antibody was tested incubating samples without the primary antibody. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole. Samples were observed at 400× magnification in an epifluorescence microscope (Leica, Wetzlar, Hesse, Germany). Images were captured with a digital camera and analyzed using the ImageJ software. The number of myofibroblasts (alpha smooth muscle actin-positive cells) was scored among 400 total cells in the area of major extracellular matrix depot.[28] Histological analysis was performed in blind.

Extracellular matrix

Seven days after abdominal surgery, the cecum was dissected. Sections from the distal third were obtained and stained with Masson's trichrome. Samples were observed at 100× and 400× magnifications in an optical microscope, and images were captured with a digital camera and analyzed using the ImageJ software. Fibrosis level was graded according to the scale proposed by Lalountas.[29] Score 0: tissue without alterations; score 1 (mild fibrosis): small area with rare collagen deposit, with no tissue connections; score 2 (moderate fibrosis): large area with scarce collagen deposit, with two tissues connected; and score 3 (severe fibrosis): extensive area with large collagen deposit, with more than two tissues connected (Fig. S2, Supporting Information). Histological analysis was performed in blind.

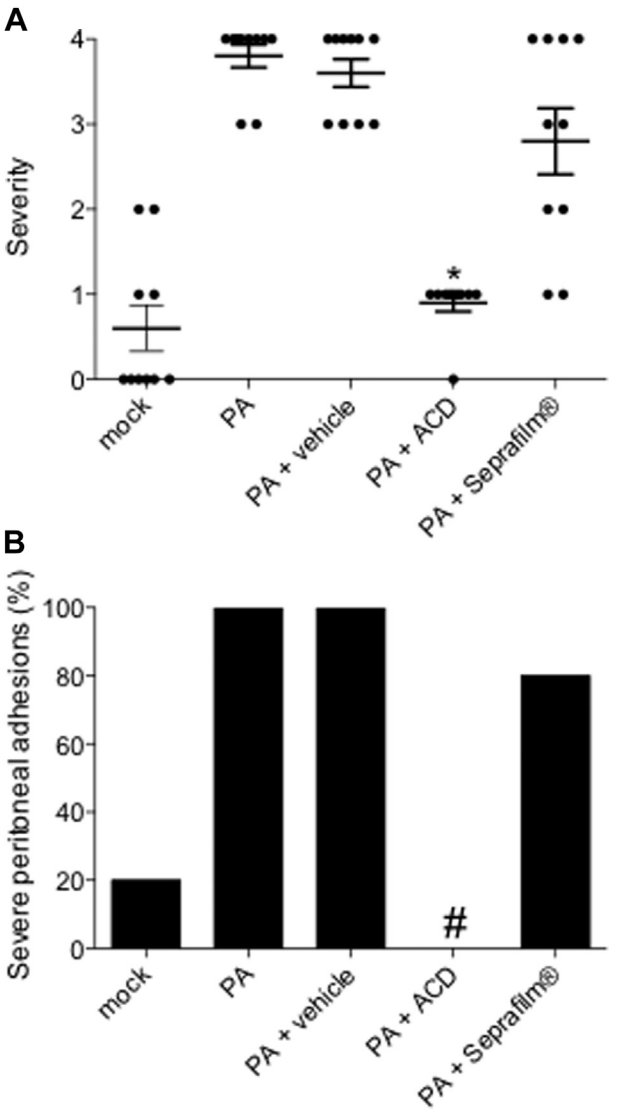

Fig. 2 − (A) Severity of peritoneal adhesions. Seven days after abdominal surgery, mice subjected to laparotomy (mock) and cecal erosion (PA) receiving the vehicle (PA + vehicle), ACD (PA + ACD), or Seprafilm (PA + Seprafilm) were euthanized, and peritoneal adhesions were assessed macroscopically; $n = 10$ per group. $^{*}P < 0.05$ in Dunn's posttest against the group PA. (B) Frequency of animals presenting severe peritoneal adhesions (grade ≥ 2) in the aforementioned experimental groups; $n = 10$ per group. $^{\#}P < 0.001$ in Fisher's exact test against the group PA.

Statistical analysis

Each experimental group included at least five animals randomly picked from at least five different litters.

Data are presented as mean ± standard error of the mean. Statistical significance of intergroup differences was determined using the Kruskal–Wallis tests and Dunn's posttest, excepting data shown in Figure 2B where Fisher's exact test was used. $P < 0.05$ was considered as statistically significant.

Results

ACD administered at the end of abdominal surgery prevents the formation of clinically relevant peritoneal adhesions

While half of the laparotomized animals (mock) developed peritoneal adhesions, all mice in which cecum was eroded (PA) presented them (Fig. 2A). Any tested intervention precluded the development of peritoneal adhesions. In animals untreated or receiving the vehicle, they scored 3 to 4, whereas in mice treated with ACD, peritoneal adhesions scored 1 ($P < 0.0001$). In animals treated with Seprafilm, peritoneal adhesions scored 1 to 4. Since clinically relevant peritoneal adhesions are the severe ones, the frequency of animals that developed adhesions scoring 2 or higher was calculated. As shown in Figure 2B, the only intervention that completely avoided the appearance of clinically relevant peritoneal adhesions was the administration of ACD ($P < 0.05$). Seprafilm impaired the development of severe peritoneal adhesions only in 25% of the animals which received it.

As the comparator showed low efficacy than ACD in the prevention of the development of peritoneal adhesion, the PA + Seprafilm group was not further studied.

ACD reduces the inflammation that precedes peritoneal adhesion formation

Abdominal inflammatory status was assessed according to the levels of proinflammatory cytokines in the peritoneal fluid, leukocyte infiltration in the cecum, and macrophages abundance in the peritoneal cavity.

One day after abdominal surgery, no significant differences were observed among experimental groups for IL1 beta (Fig. 3A). Compared with the mock group, the levels of TNF-alpha and INF-gamma were increased in the PA group (38.1 ± 6.4 pg/mL *versus* 112.4 ± 15.9 pg/mL, $P < 0.05$ and 300.1 ± 20.1 pg/mL *versus* 483.6 ± 55.4 pg/mL, $P < 0.05$, respectively). The administration of the vehicle did not prevent the augmentation of these proinflammatory cytokines. ACD inhibited the rise of both TNF-alpha (PA: 112.4 ± 15.9 *versus* PA + ACD: 33.7 ± 10.4 pg/mL, $P < 0.05$) and INF-gamma (PA: 483.6 ± 55.4 *versus* PA + ACD: 374.5 ± 53.8 pg/mL, $P < 0.05$). Three days after abdominal surgery, TNF-alpha was undetectable in all samples analyzed. Regarding INF-gamma, animals receiving ACD showed levels similar to controls and significantly lower than those found in untreated mice (PA: 260.7 ± 26.6 *versus* PA + ACD: 67.3 ± 6.9 pg/mL, $P < 0.001$; Fig. S4, Supporting Information).

One day after abdominal surgery, TNF-alpha was undetectable in the blood of all animals, and INF-gamma was increased in the PA group compared with the mock group (110.4 ± 18.7 *versus* 38.6 ± 9.6 pg/mL, $P < 0.05$; Fig. 3B). Although the vehicle did not modify the augmentation of this cytokine, ACD prevented its rise (PA: 110.4 ± 18.7 *versus* PA + ACD: 35.3 ± 6.7 pg/mL, $P < 0.05$). Three days after abdominal surgery, circulating INF-gamma became undetectable in all the experimental groups.

One day after abdominal surgery, an important infiltration of PMN and mononuclear cells was observed in the mesothelium of animals in which adhesions were induced (mock: 0.6 ± 0.4 *versus* PA: 6.4 ± 0.9 PMN cells/mm of mesothelium, $P < 0.001$ and mock: 1.4 ± 0.8 *versus* PA: 36 ± 4 mononuclear cells/mm of mesothelium, $P < 0.01$; Fig. 3C). The administration of the vehicle resulted in lower PMN but not mononuclear cell infiltration. ACD significantly reduced the infiltration of both the cell types (2.8 ± 0.7 PMN cells/mm of mesothelium and 16 ± 2.1 mononuclear cells/mm of mesothelium). Three days after abdominal surgery, only the administration of ACD prevented leukocyte infiltration (PA + vehicle: 4 ± 0.7 *versus* PA + ACD: 0.8 ± 0.3 PMN cells/mm of mesothelium, $P < 0.05$ and PA + vehicle: 38.2 ± 4.7 *versus* PA + ACD: 10 ± 3.1 mononuclear cells/mm of mesothelium, $P < 0.05$; Fig. S5, Supporting Information).

Three days after abdominal surgery, peritoneal macrophages were thrice abundant in the abdominal cavity of animals in which adhesions were induced, compared to controls (PA: 6.5 ± 0.2 *versus* mock: 2.3 ± 0.3 × 10^6 peritoneal macrophages/mL, $P < 0.05$; Fig. 3D). The vehicle did not modify the number of peritoneal macrophages. The administration of ACD resulted in significantly lower macrophages in the abdominal cavity (1.3 ± 0.1 × 10^6 peritoneal macrophages/mL, $P < 0.05$).

ACD increases the fibrinolysis that precludes peritoneal adhesion formation

Three days after abdominal surgery, fibrinolytic tone was determined according to the level of D-dimer in the peritoneal fluid. The administration of ACD resulted in a significant increase in the levels of fibrin degradation product (PA: 384.9 ± 39.9 *versus* PA + ACD: 836.7 ± 33.1 pg/mL, $P < 0.05$; Fig. 4A).

One and 3 days after abdominal surgery, fibrin deposits were found in the PA group (Fig. 4B). While the vehicle did not change their extension, ACD significantly reduced it (PA: 18.2 ± 4.7 *versus* PA + ACD: 2.9 ± 1.2 %, $P < 0.05$).

ACD reduces fibrogenesis in peritoneal adhesions

Seven days after abdominal surgery, myofibroblasts were detected in the adhesion tissue of all animals, except in those from the mock group (Fig. 5A). Compared with the untreated animals, those receiving ACD showed lesser infiltrating myofibroblasts (7.4 ± 1.3 *versus* 0.8 ± 0.4, $P < 0.05$).

Seven days after abdominal surgery, the animals in the mock group did not show fibrosis and those in the PA and PA + vehicle groups showed fibrosis grade 2 to 3 (Fig. 5B). Animals which received ACD did not show fibrosis (4/5) or it

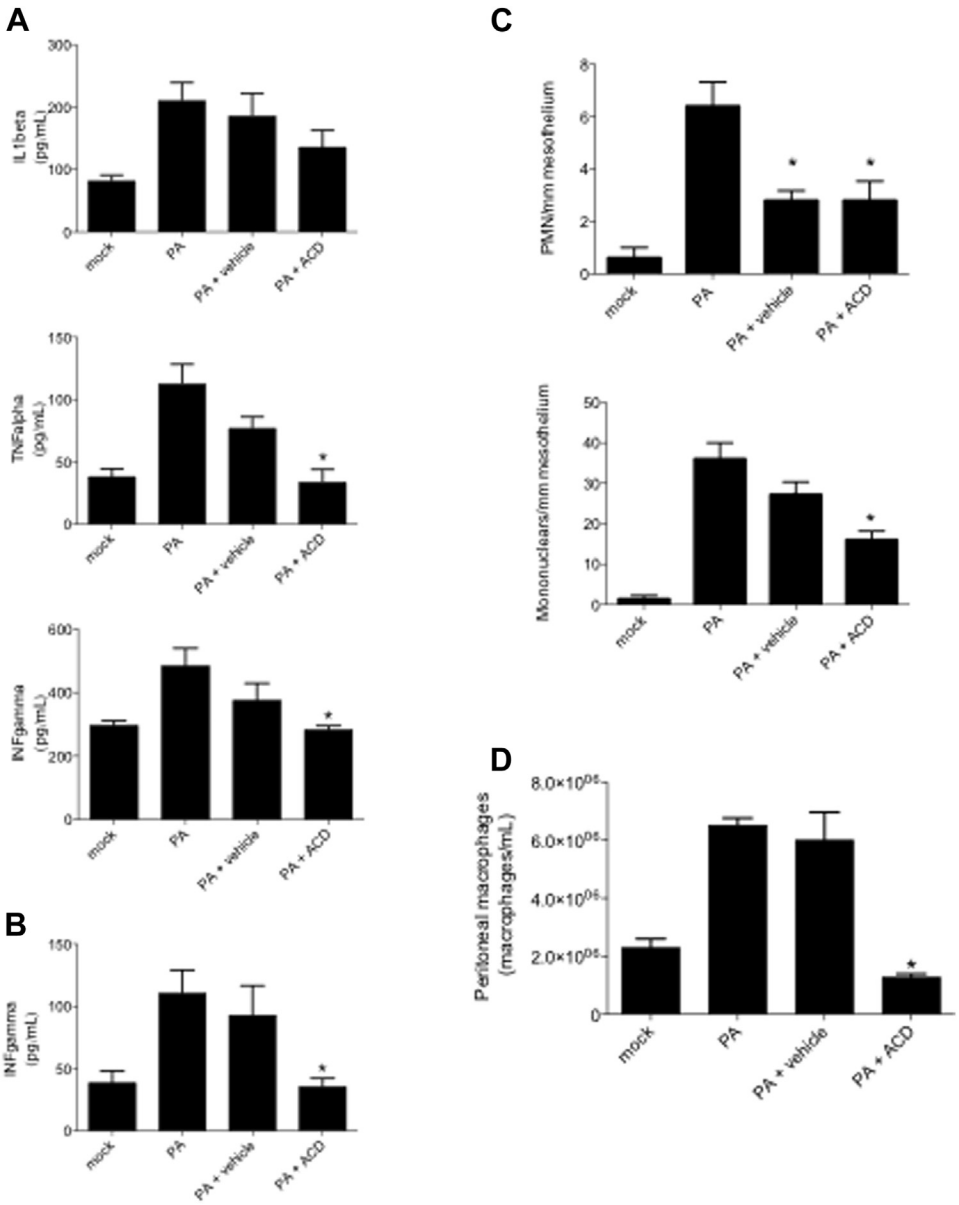

Fig. 3 – (A) Local proinflammatory cytokines levels. One day after abdominal surgery, mice subjected to laparotomy (mock) and cecal erosion (PA) receiving the vehicle (PA + vehicle) or ACD (PA + ACD) were euthanized, peritoneal fluid was collected, and proinflammatory cytokines were assessed; $n = 5$ per group. *$P < 0.05$ in Dunn's posttest against the group PA. (B) Systemic INF-gamma levels. One day after abdominal surgery, blood was collected from aforementioned animals and INF-gamma was assessed; $n = 5$ per group. *$P < 0.05$ in Dunn's posttest against the group PA. (C) Leukocyte infiltration. One day after abdominal surgery, cecum was procured from aforementioned animals, and PMN and mononuclear cells infiltrating the mesothelium were assessed; $n = 5$ per group. *$P < 0.05$ in Dunn's posttest against the group PA. (D) Macrophages in peritoneal cavity. Three days after abdominal surgery, peritoneal fluid was collected from aforementioned animals, and macrophages were assessed; $n = 5$ per group. *$P < 0.05$ in Dunn's posttest against the group PA.

was grade 1 (1/5). The observed differences between the PA and PA + ACD groups were statistically significant (2.6 ± 0.3 *versus* 0.2 ± 0.2, $P < 0.05$, respectively).

The antiadhesive effect of ACD is conserved among different MSC donors

ACDs produced from MSCs of three different donors (total protein ranging from 13.7 to 20.3 μg/μL) equally avoided the appearance of clinically relevant peritoneal adhesions (Fig. S3, Supporting Information).

Discussion

The animal model used in the current research replicates the causes and the pathophysiology of peritoneal adhesion development. While laparotomized animals (mock)

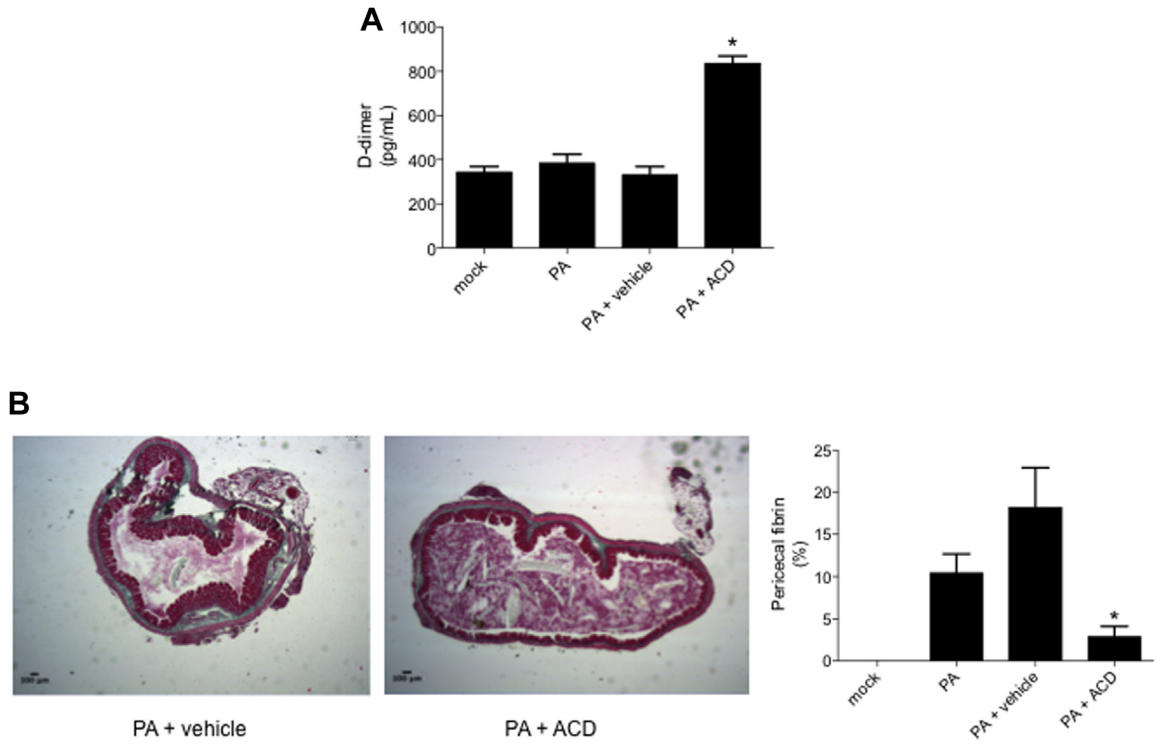

Fig. 4 – (A) D-dimer levels. Three days after abdominal surgery, mice subjected to laparotomy (mock) and cecal erosion (PA) receiving the vehicle (PA + vehicle) or ACD (PA + ACD) were euthanized, peritoneal fluid was collected, and D-dimer was assessed; $n = 5$ per group. *$P < 0.05$ in Dunn's posttest against the group PA. (B) Pericecal fibrin deposit. Three days after abdominal surgery, cecum was procured from aforementioned animals, and pericecal fibrin deposits were assessed. Bar = 100 μm. $n = 5$ per group. *$P < 0.05$ in Dunn's posttest against the group PA. (Color version of figure is available online.)

sporadically developed minor adhesive bands, all animals in which cecum was eroded (PA) developed at least one thick and wide peritoneal adhesion, which binds organs to the abdominal wall or among them. Also, an early and robust inflammatory response is mounted, leading to the infiltration of cells able to consolidate a fibrous tissue that lasts for at least 3 mo (data not shown).

In the animal model selected, we showed that the administration of a single dose of ACD at the end of the abdominal surgery prevents the formation of clinically relevant peritoneal adhesions. Also, we proved empirically that ACD is superior to the, so far, best available product for prevention of peritoneal adhesions. It is known that the main difficulty for Seprafilm to be efficient is its application that is tricky and time consuming.[10] In contrast, ACD does not require any special manipulation as it only needs to be perfused into the abdominal cavity. Actually, more complex formulation threatens its efficiency as we have proven that aerosolization makes results less accurate (data not shown). Another benefit of ACD is that its effect does not depend on MSC donor particularities, at least if they are young and healthy subjects.

The antiadhesive effect of ACD might be because of the volume increase or local chilling secondary to the instillation of a liquid.[25] We proved that none of them is the action mechanism of ACD because the vehicle failed to prevent the formation of peritoneal adhesions, showed a marginal and transient anti-inflammatory effect, and modified neither fibrinolysis nor the fibrogenesis. Therefore, the active principles of ACD must be MSC secretion products, including anti-inflammatory factors such as IL10; fibrinolytic factors such as tissue-type plasminogen activators and metalloproteinase; and antifibrogenetic factors such as hepatocyte growth factor and adrenomedullin.[13-20] The presence of all these factors in ACD allows hypothesizing that each subgroup of components disrupts independently a step in the pathogenic process. Nevertheless, because of the interdependence of the mechanisms associated with the development of peritoneal adhesions, it is also possible that the anti-inflammatory activity of ACD may be sufficient to preclude the onset of scar tissue. However, in preclinical studies, it has been shown that local or systemic administration of anti-inflammatories results in a decrease in proinflammatory cytokine levels and in leukocyte infiltration but did not avoid the development of peritoneal adhesions.[21,22,24,25] These results, obtained with anti-inflammatory concentrations that may be toxic for humans, suggest that interrupting the inflammatory process is necessary but not sufficient to prevent the formation of peritoneal adhesions. Further research is required to know both the active principles and action mechanisms of ACD.

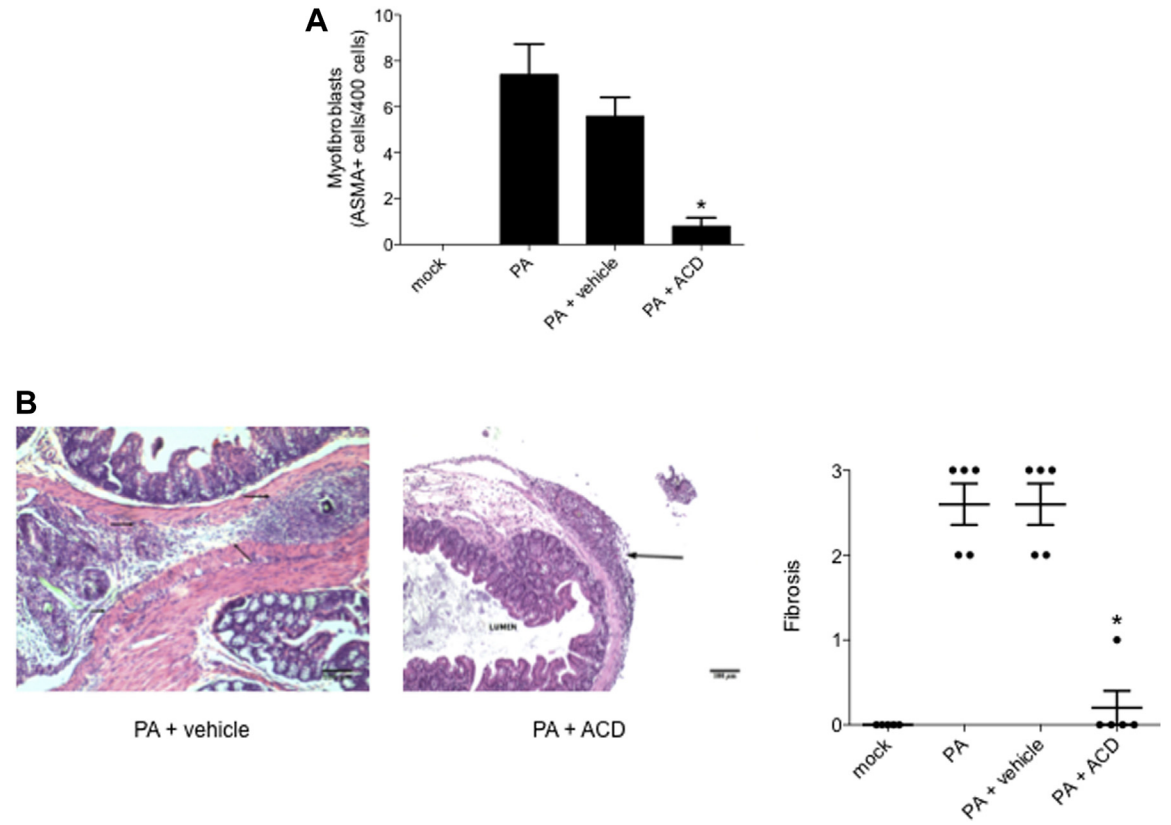

Fig. 5 – (A) Myofibroblasts infiltration. Seven days after abdominal surgery, mice subjected to laparotomy (mock) and cecal erosion (PA) receiving the vehicle (PA + vehicle) or ACD (PA + ACD) were euthanized, adhesive tissue was procured, and myofibroblasts (alpha smooth muscle actin-positive cells) were assessed; $n = 5$ per group. *$P < 0.05$ in Dunn's posttest against the group PA. (B) Fibrosis grade. Seven days after abdominal surgery, cecum was procured from aforementioned animals, and fibrous tissue was graded. Bar = 25 μm. Arrow shows fibrotic tissue limit; $n = 5$ per group. *$P < 0.05$ in Dunn's posttest against the group PA. (Color version of figure is available online.)

It is also necessary to improve some technological aspects, that is, the adjustment of the volume to be used in humans, because, scaling proportionally, almost 1 L of ACD would be necessary for a person who weights 70 kg. Alternatively, it would be desirable to concentrate the ACD. Lyophilization is not an alternative because it results in a loss of effect (data not shown).

Use of ACD instead of MSCs for prevention purpose has clear advantages because it minimizes the biological risks of cell therapy (disease transmission and neoplasies); decreases production, distribution, and storage costs; and makes the scaling and massification more attainable.[30]

To our knowledge, we show for the first time that the bioactives secreted by human MSCs prevent the formation of peritoneal adhesions. Further preclinical and clinical studies are needed to sustain the prophylactic use of ACD as an antiadhesive agent.

Conclusions

ACD administered at the end of abdominal surgeries prevents the formation of peritoneal adhesions due to the modulation of inflammatory, fibrinolytic, and fibrogenic processes.

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Authors' contributions: D.R. designed the study, performed the experiments, analyzed data, wrote the manuscript, and read and approved the final manuscript. P.C. designed the study, supervised all procedures, analyzed data, revised the manuscript, and read and approved the final manuscript.

Disclosures

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jss.2017.11.018.

REFERENCES

1. ten Broek RP, Issa Y, van Santbrink EJ, et al. Burden of adhesions in abdominal and pelvic surgery: systematic review and met-analysis. *BMJ*. 2013;347:f5588.
2. Arung W, Meurisse M, Detry O. Pathophysiology and prevention of postoperative peritoneal adhesions. *World J Gastroenterol*. 2011;17:4545–4553.
3. Alpay Z, Saed GM, Diamond MP. Postoperative adhesions: from formation to prevention. *Semin Reprod Med*. 2008;26:313–321.
4. Diamond MP, Freeman ML. Clinical implications of postsurgical adhesions. *Hum Reprod Update*. 2001;7:567–576.
5. Reinke JM, Sorg H. Wound repair and regeneration. *Eur Surg Res*. 2012;49:35–43.
6. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. *Physiol Rev*. 2003;83:835–870.
7. Alan Stevens JL. *Anatomía Patológica*. Segunda ed. España: Elsevier España; 2001.
8. Chegini N. TGF-beta system: the principal profibrotic mediator of peritoneal adhesion formation. *Semin Reprod Med*. 2008;26:298–312.
9. Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med*. 2012;18:1028–1040.
10. Di Saverio S, Coccolini F, Galati M, et al. Bologna guidelines for diagnosis and management of adhesive small bowel obstruction (ASBO): 2013 update of the evidence-based guidelines from the world society of emergency surgery ASBO working group. *World J Emerg Surg*. 2013;8:42.
11. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem*. 2006;98:1076–1084.
12. Kim HS, Choi DY, Yun SJ, et al. Proteomic analysis of microvesicles derived from human mesenchymal stem cells. *J Proteome Res*. 2012;11:839–849.
13. Reitamo S, Remitz A, Tamai K, et al. Interleukin-10 modulates type I collagen and matrix metalloprotease gene expression in cultured human skin fibroblasts. *J Clin Invest*. 1994;94:2489–2492.
14. Sato Y, Ohshima T, Kondo T. Regulatory role of endogenous interleukin-10 in cutaneous inflammatory response of murine wound healing. *Biochem Biophys Res Commun*. 1999;265:194–199.
15. Yamamoto T, Eckes B, Krieg T. Effect of interleukin-10 on the gene expression of type I collagen, fibronectin, and decorin in human skin fibroblasts: differential regulation by transforming growth factor-beta and monocyte chemoattractant protein-1. *Biochem Biophys Res Commun*. 2001;281:200–205.
16. Neuss S, Schneider RK, Tietze L, et al. Secretion of fibrinolytic enzymes facilitates human mesenchymal stem cell invasion into fibrin clots. *Cells Tissues Organs*. 2010;191:36–46.
17. Li L, Zhang Y, Li Y, et al. Mesenchymal stem cell transplantation attenuates cardiac fibrosis associated with isoproterenol-induced global heart failure. *Transpl Int*. 2008;21:1181–1189.
18. Li L, Zhang S, Zhang Y, et al. Paracrine action mediate the antifibrotic effect of transplanted mesenchymal stem cells in a rat model of global heart failure. *Mol Biol Rep*. 2009;36:725–731.
19. Cantinieaux D, Quertainmont R, Blacher S, et al. Conditioned medium from bone marrow-derived mesenchymal stem cells improves recovery after spinal cord injury in rats: an original strategy to avoid cell transplantation. *PLoS One*. 2013;8:e69515.
20. Chen L, Tredget EE, Wu PY, et al. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS One*. 2008;3:e1886.
21. Dinarvand P, Farhadian S, Seyedjafari E, et al. Novel approach to reduce postsurgical adhesions to a minimum: administration of losartan plus atorvastatin intraperitoneally. *J Surg Res*. 2013;181:91–98.
22. Yilmaz HG, Tacyildiz IH, Keles C, et al. Micronized purified flavonoid fraction may prevent formation of intraperitoneal adhesions in rats. *Fertil Steril*. 2005;84:1083–1088.
23. Solis EA, Bouvet BR, Brufman AS, et al. The possible macrophage role in seminal fluid. *Actas Urol Esp*. 2003;27:185–189.
24. Dinarvand P, Hassanian SM, Weiler H, et al. Intraperitoneal administration of activated protein C prevents postsurgical adhesion band formation. *Blood*. 2015;125:1339–1348.
25. Fang CC, Chou TH, Lin GS, et al. Peritoneal infusion with cold saline decreased postoperative intra-abdominal adhesion formation. *World J Surg*. 2010;34:721–727.
26. Cataldo DD, Tournoy KG, Vermaelen K, et al. Matrix metalloproteinase-9 deficiency impairs cellular infiltration and bronchial hyperresponsiveness during allergen-induced airway inflammation. *Am J Pathol*. 2002;161:491–498.
27. Zhang L, Ding WY, Wang ZH, et al. Early administration of trimetazidine attenuates diabetic cardiomyopathy in rats by alleviating fibrosis, reducing apoptosis and enhancing autophagy. *J Transl Med*. 2016;14:109.
28. Cha SI, Groshong SD, Frankel SK, et al. Compartmentalized expression of c-FLIP in lung tissues of patients with idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 2010;42:140–148.
29. Lalountas M, Ballas KD, Michalakis A, et al. Postoperative adhesion prevention using a statin-containing cellulose film in an experimental model. *Br J Surg*. 2012;99:423–429.
30. Baglio SR, Pegtel DM, Baldini N. Mesenchymal stem cell secreted vesicles provide novel opportunities in (stem) cell-free therapy. *Front Physiol*. 2012;3:359.