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

Daniel Rojo, MD,[a,b] *and Paulette Conget, PhD*[a,*]

[a] *Centro de Medicina Regenerativa, Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina Clínica Alemana Universidad del Desarrollo, Santiago, Chile*

[b] *Doctorado en Biotecnología, Universidad de Santiago de Chile, Santiago, Chile*

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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

* *Corresponding author*. Av. Las Condes 12 438, Santiago, Chile. Tel.: +56 2 2327 9302.
E-mail address: pconget@udd.cl (P. Conget).

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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).