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Intraperitoneal application of phospholipids for the prevention of postoperative adhesions: a possible role of myofibroblasts

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ABSTRACT

Background: Peritoneal adhesions, organized as fibrous bands after abdominal surgery, are related with considerable morbidity and repeated hospitalization. Phospholipids, natural constituents of the peritoneal fluid, seem to display excellent antiadhesive properties. The aim of this study was to investigate whether intraperitoneal application of phospholipids is capable of reducing postoperative adhesions and the possible underlying mechanisms.

Materials and methods: Twenty male Wistar rats were subjected to a midline laparotomy and a standard peritoneal and cecum abrasion trauma. Before laparotomy closure, a bolus of 3 mL of phospholipids (12 mg/mL) or NaCl (placebo) was given intraperitoneally. Seven days later, the quality and the quantity of adhesions, as well as serum proinflammatory and/or profibrotic mediators, were blindly assessed. Human colonic subepithelial myofibroblasts were isolated from normal controls and cultured with transforming growth factor-β1 (TGFβ1, 5 ng/mL) in the presence of phospholipids (30–300 μg/mL). Collagen production in culture supernatants and migratory activity of myofibroblasts were also assessed.

Results: Phospholipids reduced intra-abdominal adhesions ($P < 0.001$), with respect to their intensity and area, and serum levels of cytokines (interleukin 1β, interleukin 6, platelet-derived growth factor-1, and TGFβ1) compared with placebo-treated rats. Stimulation of myofibroblasts with TGFβ1 significantly increased ($P < 0.001$) the basic collagen production. The presence of phospholipids significantly reduced ($P < 0.001$) both the TGFβ1 induced and the basic collagen production. Using a wound healing assay, phospholipids were found to reduce the basic and the TGFβ1-induced migration of myofibroblasts in a concentration-dependent manner.

Conclusions: Intraperitoneal phospholipids might be involved in the prevention of postoperative adhesions formation via the reduction of proinflammatory and/or profibrotic mediators and by inhibiting fibrogenic properties of mesenchymal cells.

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1. Introduction

Postoperative peritoneal adhesions formation is frequent and serious sequelae after gastrointestinal and pelvic surgery, remaining the leading cause of chronic abdominal pain and of small bowel obstruction, in the western world [1]. Because the pathophysiology of adhesion formation is well known, insufficient fibrinolysis at the site of visceral and parietal peritoneum injury resulting in incomplete resorption of local fibrinous deposits [2,3] decades of products and devices, based either on hydroflotation or on the barrier phenomenon [4], have been tested experimentally and in humans. Thus far, no substance has been found consistently successful to achieve general acceptance for routine clinical usage.

The ideal material should have the following characteristics: antiadhesive, highly biocompatible, biodegradable, resorbable, safe, noninflammatory, nonimmunogenic, adherent to the traumatized surface, persisting during the critical remesothelialization phase, effective on oozing surfaces, applicable through the laparoscope, staying in place without sutures or staples, be rapidly and easily applied, and not too expensive [5–7]. Moreover, for the cases of surgery that conclude to an anastomosis, the ideal method should not intervene with the anastomotic healing process.

About three decades ago, phospholipids, the main constituents of the biological membranes, attracted the interest of the investigators as a substance naturally being in the peritoneal cavity; phosphatidylcholine, isolated from the peritoneal effluents in continuous ambulatory peritoneal dialysis patients [8,9], was found to have the capacity to reduce surface tension and promote water repellency. They demonstrate an excellent lubricating action [10], by covering the whole visceral and parietal peritoneum surface, acting as liquid barrier separating apposite areas of peritoneal surfaces by a very thin, membrane-like film, reducing thus the adhesion formation in different settings, including generalized peritonitis [11–16]. In a more recent experimental work in a rabbit model subjected to laparotomy, abrasion damage of parietal and visceral peritoneum, jejunal anastomosis and electrocautery incision of the liver, doses of up to 120-mg/kg body weight were used; in this model, the efficacy of phospholipids in adhesion prevention as well as the safety of the agent were confirmed by means of the findings of uncompromised healing of the anastomosis, laparotomy wounds, and liver incisions [16]. However, information on the mechanisms of the antiadhesive action of phospholipids is lacking.

The present study was undertaken to investigate possible mechanisms by which phospholipids prevent adhesion formation (1) *in vivo*, by assessing the extent of adhesions and some biological parameters of the fibrinogenic and/or fibrinolytic pathway, and (2) in cell cultures, by assessing the prevention of myofibroblast's overgrowth and collagen deposition.

2. Materials and methods

2.1. Materials

For cell culture treatments, human active recombinant transforming growth factor-β1 (TGFβ1) was purchased from R&D Systems (Abingdon, United Kingdom). Vitalipid, which contains 12-mg/mL purified egg phospholipids that consist of 83% phosphatidylcholine, 15% phosphatidylethanolamine, 1% sterols, and 1% other phospholipids, was used as a phospholipid source, and it was purchased from Fresenius Kabi Hellas S.A. (Athens, Greece). The Sircol collagen assay was purchased from Biocolor Ltd (Carrickfergus, United Kingdom).

2.2. Animals

Twenty male Wistar rats weighing 250–300 g were housed together in stable laboratory conditions with a 12-h light–dark cycle and were given free access to tap water and standard pellet rat food until 24 h before the time of operation. All the rats were of the same gender to minimize any sex factors that could potentially influence results. The experiment was performed at the Surgical Research Laboratory of the AHEPA University Hospital. The experimental protocol was approved by the Department of Animal Care and Use Committee of the Greek Ministry of Agriculture and adhered to the European Community Guiding Principles for the Care and Use of Animals.

2.3. Experimental settings

At the end of the adaptation period, after withholding food but not water for 24 h, each rat was subjected to 50 mg/kg of ketamine hydrochloride intramuscular anesthesia (Ketalar; Parke-Davis Pty. Ltd. Caringbah, NSW, Australia, 50 mg/mL) and 5 mg/kg of xylazine (Xylazine-20 Injection; Butler Company, Columbus, OH). A midline laparotomy was then performed to all rats followed by a standard peritoneal and cecum abrasion trauma. In detail, a 1-cm^2 area of the cecum and the right side of the abdominal wall opposite the cecum were brushed five times with a medium sandpaper nail brush, thus the serosa was denuded and petechiae developed. Before the last stitch of abdominal closure, the rats were randomly divided into two groups, the control-treated (10 rats) and the phospholipids-treated (10 rats). The phospholipids-treated rats received 120 mg/kg of phospholipids (12 mg/mL solution, 2.76 ± 0.14 mL volume administered, 33.18 ± 1.75 [range 30–36 mg, median 33.3 mg] dose per animal), whereas the control group received a volume of 3 mL of 0.9% saline solution instilled into the abdominal cavity. After recovery from anesthesia, rats were individually caged in Plexiglas (Innovive Inc, San Diego, CA) cages with bedding and allowed to receive tap water and standard pellet rat food *ad libitum* for the next 7 d. Seven days thereafter, rats were anesthetized, as previously, and an extended U-shaped laparotomy incision was performed for adhesion inspection and assessment.

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