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Antiadhesive and anti-inflammatory effects of pirfenidone in postoperative intra-abdominal adhesion in an experimental rat model

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ABSTRACT

Background: Pirfenidone (PF) is a potent antifibrotic and anti-inflammatory agent. We investigated the protective effect of PF against postoperative intra-abdominal adhesions.
Material and methods: Thirty male Sprague–Dawley rats were divided into three groups ($n = 10$ in each group). In group 1 (control), adhesion induction was performed by cecal abrasion, and no treatment was administered. In group 2 (vehicle), for 2 wk after adhesion induction, 0.4%-carboxymethylcellulose was administered by gavage. In group 3 (PF treatment), for 2 wk after adhesion induction, 500-mg/kg/d PF was administered by gavage. On the 15th postoperative day, the animals were killed, and cecal and peritoneal tissues were excised. The adhesions were graded macroscopically. The protein concentrations and mRNA expression levels of the following genes were measured in the tissues: matrix metallopeptidase-9 (MMP-9); tissue inhibitor of metalloproteinase-1 (TIMP-1); tumor necrosis factor-alpha (TNF-α); and transforming growth factor-beta 1 (TGF-β1). The tissue samples were also evaluated histopathologically.
Results: Macroscopic and histopathologic evaluation showed that PF-reduced adhesion and inflammation ($P < 0.001$, $P = 0.004$, respectively). Pretreatment with PF-reduced TIMP-1, TNF-α, and TGF-β1 protein concentrations ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively) and mRNA expression levels ($P = 0.030$, $P = 0.005$, and $P = 0.016$, respectively) and increased MMP-9 protein concentrations ($P < 0.001$) and mRNA expression ($P = 0.021$).
Conclusions: The findings of this study suggest that PF can be used as a protective agent to prevent the development of peritoneal adhesions and inflammation during the postoperative period.

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1. Introduction

Postoperative adhesions are the pathologic fibrotic bands that develop between the peritoneal surfaces in the peritoneal cavity. Although most adhesions develop after abdominal surgery, they can develop due to pathologic conditions, such as peritonitis, malignancy, endometriosis, pelvic inflammatory disease, and long-term peritoneal dialysis [1]. Adhesion formation is a complex process involving cellular, biochemical, and immunologic factors. Impairment of the delicate balance between fibrin formation and fibrinolysis causes an extensive inflammatory response and adhesion formation [2]. The most important surgical complication that can develop due to peritoneal adhesions is intestinal obstruction. Other complications that can develop include infertility, chronic abdominal pain, and difficulties in subsequent surgical interventions [3]. Therefore, prevention or reduction of intra-abdominal adhesions is a highly important goal to minimize the above-mentioned complications. Although many treatment methods have been described to prevent the development of postoperative peritoneal adhesions, and there have been recent developments in surgical techniques, postoperative adhesions are still a major clinical problem [4,5].

Pirfenidone (5-methyl-1-phenyl-2-[1H]-pyridone, PF) is a novel small compound with anti-inflammatory, antioxidant, and antifibrotic effects that was initially developed as an antihelminthic and antipyretic agent [6–9]. Experimental animal models of pulmonary fibrosis have shown that PF exerts its effects by reducing the levels of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1, interleukin-1β, and interleukin-6, by downregulating the transcription of profibrotic growth factors, including transforming growth factor-β (TGF-β), and by reducing lipid peroxidation and oxidative stress [8–12]. Clinical studies in idiopathic pulmonary fibrosis have demonstrated the effectiveness of PF in extending survival time and improving pulmonary function [13–15]. PF has also been shown to have beneficial effects in renal and liver fibrosis, and it exerts those effects by upregulating the transcription of matrix metalloproteinases (MMPs) and downregulating the transcription of tissue inhibitors of metalloproteinases (TIMPs) [16,17]. PF is highly soluble in aqueous solutions and is able to move through cell membranes without requiring a receptor. When administered orally, PF is easily absorbed in the gastrointestinal tract, reaching most tissues and crossing the blood-brain barrier. After oral administration, PF reaches its maximum level in the blood after 1 to 2 h and is excreted in the urine. Regarding the safety of PF, most studies have reported no significant toxicity attributable to the drug at doses of approximately 2500 mg/d [7,18].

Therefore, in this study, we investigated the anti-inflammatory and antiadhesive effects of PF in postoperative intraabdominal adhesions in an experimental rat model using biochemical (matrix metallopeptidase-9 (MMP-9); tissue inhibitor of metalloproteinase-1, TIMP-1; TNF-α; and TGF-β1 protein levels), molecular (MMP-9, TIMP-1, TNF-α, and TGF-β1 mRNA expression levels), and histopathologic analyses.

2. Materials and methods

This study was carried out at the Experimental Animals Research Center of Dumlupinar University. The study experiments were approved by the Local Ethics Committee for Experiments on Animals of Dumlupinar University (No: 2014.11.01). All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the Institute of Laboratory Animal Resources Commission on Life Sciences National Research Council [19].

2.1. Animals

Male Sprague–Dawley rats (300–350 g, 12–16 wk, $n = 30$) were used in this study. All rats were housed individually in transparent polycarbonate cages with a 12-h:12-h light-dark cycle at 22 ± 2°C and were provided ad libitum access to fresh water and standard rat chow until the experiments commenced.

2.2. Chemicals

Pirfenidone was purchased from Sigma-Aldrich Co. LLC. (Sigma-Aldrich Co. LLC., St. Louis, MO) and was dissolved in carboxymethylcellulose (CMC) (Sigma–Aldrich Co. LLC., St. Louis, MO) [20].

2.3. Experimental study design

The experimental animals were randomly divided into three groups ($n = 10$). In group 1 (control), adhesion induction was performed by cecal abrasion, and no treatment was administered. In group 2 (vehicle), for 2 wk after adhesion induction, 1 mL of 0.4% CMC was administered by orogastric gavage. In group 3 (PF treatment), for 2 wk after adhesion induction, 500-mg/kg/d PF was administered by orogastric gavage [21].

2.4. Surgical procedures

Rats were weighed and then anesthetized with an intraperitoneal (i.p) injection of 10-mg/kg xylazine hydrochloride (Rompun, Bayer, Istanbul, Turkey) and 70-mg/kg ketamine (Ketalar, Pfizer, Istanbul, Turkey). After a suitable level of anesthesia was achieved, the rats were placed on a homeothermic table to maintain a stable body temperature of 37 ± 1°C. Then, the anterior abdominal wall was shaved and sterilized with povidone-iodine solution. Nonpowdered gloves were used for the experiment. A 5-cm midline incision was made in the abdomen from the xiphoid. After laparotomy, the antimesenteric border of the cecum was abraded by rubbing it with dry sterile gauze approximately 20 times until punctuate bleeding occurred in approximately a 2-cm area. A similar area of 2 × 2 cm in size in the adjacent side-wall peritoneum was excised from the abdominal wall. The viscera were replaced. The abdominal cavity was closed using continuous 3/0 polypropylene sutures. After 15 d, relaparotomy was performed, and the abdominal cavity was inspected through a U-shaped incision [22]. At the end of the experiment, the animals were killed under anesthesia. Both the cecal and peritoneal