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Neutrophils contribute to the pathogenesis of hemorrhagic cystitis induced by ifosfamide

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ABSTRACT

Ifosfamide (IFO) is an antineoplastic drug that is commonly used to treat gynecological and breast cancers. Hemorrhagic cystitis (HC) is a common side effect associated with IFO injection, which courses with neutrophil accumulation and affects 6–50% of patients depending on dose intensity. Here, we investigated the role of neutrophils in this inflammatory process. Female Swiss mice (n = 8/group) were injected with saline, IFO (400 mg/kg, i.p.), fucoidan (a P- and L-selectins inhibitor, 100 mg/kg, i.v.) or IFO + fucoidan (1–100 mg/kg) alone or combined with mesna (80 mg/kg i.p.). Another group of mice received anti-Ly6G antibody (500 μg/mouse, once daily for 2 days) for neutrophil depletion before IFO injection. In another experimental setting, animals received granulocyte colony-stimulating factor (G-CSF, 400 μg/kg), IFO (200 mg/kg), G-CSF (25–400 μg/kg, for 5 days) + IFO (200 mg/kg, i.p.) or fucoidan + G-CSF + IFO. Bladder injury was evaluated 12 h after IFO injection. IFO 400 mg/kg significantly increased visceral hyperalgesia, bladder edema, hemorrhage, vascular permeability, MPO, IL-1β and IL-6 tissue levels, and COX-2 immunostaining and expression versus the saline group ($P < 0.05$). Conversely, fucoidan (100 mg/kg) significantly attenuated these parameters compared to IFO-injected mice ($P < 0.05$). Additionally, fucoidan potentiated mesna protective effect when compared with IFO + mesna group ($P < 0.05$). Accordingly, neutrophil depletion with anti-Ly6G reduced inflammatory parameters and bladder injury compared to IFO ($P < 0.05$). In contrast, G-CSF enhanced IFO (200 mg/kg)-induced HC, which was significantly attenuated by treatment with fucoidan ($P < 0.05$). Therefore, neutrophils contribute to the pathogenesis of HC.

1. Introduction

Chemotherapy-induced hemorrhagic cystitis (HC) [1] is a common and limiting side effect in patients undergoing chemotherapy with alkylating agents, such as the oxazaphosphorines ifosfamide (IFO) and cyclophosphamide. Liver metabolism of these agents produces acrolein, which lacks anticancer activity and accumulates in the bladder, leading to HC development [2]. Patients with HC generally experience complex symptoms known as “LUTS” (lower urinary tract symptoms), which is characterized by dysuria, urinary urgency, nocturia, suprapubic pain

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and microscopic or macroscopic hematuria [1].

The average incidence of HC ranges from 18% to 40% in the absence of adequate uroprotection [3]. Prophylactic measures, including mesna, bladder irrigation and hyperhydration, are not totally capable of blocking inflammatory events as observed in clinical trials and in experimental conditions [4]. Accordingly, patients treated with standard clinical protocol of mesna show bladder mucosa microscopic alterations, such as edema, exocytosis, and hemorrhage in 100% of cases. Cystoscopic alterations are also detected in 66.7% of patients [5]. Patient recipients of preconditioning high-dose chemotherapy during bone marrow transplantation continue to exhibit severe and life-threatening HC, despite the prophylactic use of mesna [6]. Therefore, the elucidation of HC pathogenesis opens novel perspectives for more effective preventive therapeutic strategies.

A number of studies report the involvement of inflammatory mediators in the pathogenesis of HC, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), nitric oxide (NO) and platelet activating factor (PAF), which cause edema and hemorrhage [6–14]. These mediators partially contribute to bladder injury via the recruitment of neutrophils, since neutralization of these inflammatory markers reduces neutrophil accumulation in the bladder and the severity of HC [14]. Notably, the use of the chemokine receptor 2 (CXCR2) antagonist SB225002 prevents painful behaviors, bladder inflammation and voiding dysfunction during cyclophosphamide-induced cystitis in rats. Conversely, IL-8, which activates CXCR2, is an important mediator that is necessary for normal urothelial cell organization. Therefore, CXCR2 modulation may negatively impact urothelial survival [15]. Several redundant and versatile mechanisms other than IL-8 contribute to neutrophil migration to sites of inflammation [16], and P- and L-selectins may be attractive targets.

Fucoidan is a highly sulfated polysaccharide containing L-fucose groups [17]. Fucoidan has been proved to inhibit inflammation, coagulation, thrombosis, angiogenesis, cancer, oxidative-stress, nociception, and virus infections [18]. The pharmacological underlying mechanism has been explored. The anticoagulant and antithrombotic activities is associated to the direct inactivation of thrombin in the presence of heparin cofactor II [19]. Additionally, fucoidan is described to inhibit P- and L-selectin with a high affinity by preventing selectin to bind Sialyl Lewis X with a 50% inhibitory concentration (IC_{50}) of 20 nM [20].

Up-regulation of selectins on endothelium is a crucial step to initiate neutrophil recruitment. L-selectin is constitutively expressed on circulating leukocytes, while P- and *E*-selectin are rapidly expressed on the apical endothelial cell membrane after stimulation, where they bind to ligands on neutrophils [21, 22]. Transitory interaction between selectins and their ligands on polymorphonuclear cells leads to neutrophil tethering and rolling, the initial steps of the leukocyte recruitment to the inflammatory site [23]. Consequently, in the presence of fucoidan, both P- and L-selectins are blocked, causing a reduction on leukocyte migration, which abrogates the inflammatory process [24, 25].

Therefore, the role of neutrophils in the pathogenesis of ifosfamide-induced hemorrhagic cystitis was investigated by P- and L-selectins blockade with fucoidan or neutrophil depletion with anti-Ly6G monoclonal antibody.

2. Materials and methods

2.1. Animals

Female Swiss mice weighing 20–25 g (n = 8/group) were housed in the animal facility of the Department of Physiology and Pharmacology/Federal University of Ceará and the School of Medicine of Ribeirão Preto/University of São Paulo, which provided animals with appropriate sanitary conditions. The animals were housed in controlled environmental conditions (24 ± 1 °C), under a 12/12 h light/dark cycle with food and water available ad libitum. All procedures were performed in accordance with the NIH guidelines for care and use of laboratory animals. The local Ethics Committee for Animal Experiments approved all procedures (protocol number 83/2014).

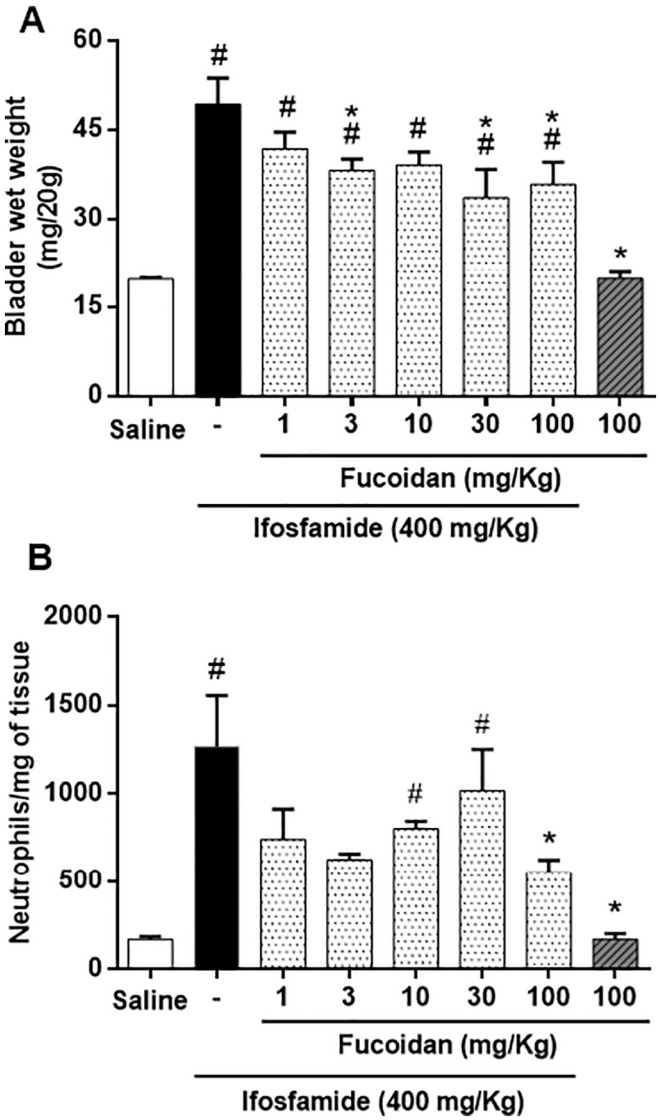

Fig. 1. Fucoidan prevents bladder edema and neutrophil infiltrate during ifosfamide-induced hemorrhagic cystitis. The mice were treated with saline (5 mL/kg i.p.), IFO (400 mg/kg, i.p.), fucoidan (100 mg/kg, i.v.) alone or fucoidan (1, 3, 10, 30 or 100 mg/kg, i.v.) 30 min before IFO. Then, bladders wet weight was measured and the myeloperoxidase activity determined. IFO significantly increased bladder wet weight (A) and neutrophil accumulation (B) compared to the saline group. Fucoidan significantly attenuated these parameters (Panels A and B) compared to the IFO group. The results are reported as means ± SEM (n = 8/group). $^{\#}P < 0.05$ vs. saline group (negative control); $^{*}P < 0.05$ vs. IFO group.

2.2. Drugs

Ifosfamide (IFO, Holoxane®, Baxter, Halle/Westfalen, Germany), Mesna (Mitexan®, Eurofarma, São Paulo, SP, Brazil.), Fucoidan® (Fucoidan from *Fucus vesiculosus*, Sigma-Aldrich, São Paulo, SP, Brazil), granulocyte colony stimulating factor (G-CSF, Filgrastrim, Genérico Biosintética, São Paulo, SP, Brazil) and anti-Ly6G monoclonal antibody (clone 1A8, catalog BE0075-1, Bio X cell, New Hampshire, USA) were purchased from commercial sources. All drugs were diluted in 0.9% saline.