International Immunopharmacology 43 (2017) 108–115

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Tripterygium wilfordii polyglycosidium ameliorates pouchitis induced by dextran sulfate sodium in rats

Senyang Gao [1], Xiaoyan Wu [1], Yingying Zhang, Kaiyu Li, Litian Wang, Gang Liu *

Department of General Surgery, Tianjin Medical University General Hospital, Tianjin, China

ARTICLE INFO

Article history:
Received 31 August 2016
Received in revised form 27 November 2016
Accepted 6 December 2016
Available online 15 December 2016

Keywords:
Tripterygium wilfordii
Dextran sulfate sodium
Pouchitis
Ileal pouch anal anastomosis
Postoperative complication

ABSTRACT

Background: The aim of this study was to investigate the therapeutic effects of *Tripterygium wilfordii* polyglycosidium (TWP) to rats with dextran sulfate sodium (DSS)-induced pouchitis and its possible mechanism.
Methods: Sprague-Dawley rats underwent surgery of ileal pouch anal anastomosis (IPAA) and pouchitis was induced by DSS. Rats were randomly divided into no intervention (NI), normal saline (NS) and TWP groups. Rats were lavaged with normal saline (3 ml/day in NS group) or TWP (12 mg/kg/day in TWP group) for 7 days. General conditions of animals and histopathological examinations were evaluated. Interleukin (IL)-1β, IL-6, IL-10, and tumor necrosis factor (TNF)-α mRNA expression was measured. Levels of occludin and Zo-1 proteins were measured by immunohistochemistry. In addition, ALT and AST were assessed.
Results: TWP significantly attenuated the symptoms of pouchitis characterized by body weight loss, diarrhea, and bloody stool. Furthermore, TWP diminished histological damage compared with other groups. There was a significant reduction in levels of IL-1β, IL-6, and TNF-α, as well as an increase in IL-10 in the TWP group. The expression of tight junction proteins occludin and Zo-1 were increased in the TWP group. There were no statistical differences in serum ALT and AST among the three groups.
Conclusions: TWP significantly ameliorated pouchitis and inhibited the production of IL-1β, IL-6, and TNF-α as well as increased the levels of IL-10, occludin, and Zo-1 protein in rats. These findings suggest TWP might be a potential therapeutic agent for patients with pouchitis.

1. Introduction

Restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA) is currently the surgical treatment of choice for medically refractory ulcerative colitis (UC), as well as for patients with familial adenomatous polyposis (FAP) [1]. Although the ileal pouch serves as a reservoir for the stool and improves functional outcomes [2], pouchitis, an inflammation of this conduit, is a common complication in patients undergoing surgery for UC, and was reported to occur in approximately 50% of patients [3]. Symptoms of pouchitis include diarrhea, increased stool frequency, abdominal cramping, fecal urgency, tenesmus, and incontinence [4]. The pathogenesis of pouchitis remains incompletely understood but might be caused by altered mucosal metabolism [5], ischemia [6], bid acid cytotoxicity [7], recurrence of UC, and genetic susceptibility [8]. IL-1 receptor antagonist (IL-1 RA) and TNF are involved in the regulation of epithelial physiology and defense, suggesting mucosal barrier function may have a pathogenic role in pouchitis [9].

TWP is a traditional Chinese medicine extracted from the roots of *Tripterygium wilfordii* Hook F. (TWhF), and was reported to be therapeutically efficacious in the treatment of autoimmune and inflammation-related diseases such as rheumatoid arthritis, ulcerative colitis, and Crohn's disease [10,11]. Triptolide is the major active component of TWP [12] and it is superior to placebo in inducing remission and preventing clinical postoperative recurrence in inflammatory bowel disease (IBD) patients [13]. WU et al. reported that triptolide reduced intestinal permeability and protected the intestinal mucosal barrier function by inhibiting TNF-α–induced tight junction protein changes in epithelial cells [14]. Therefore, TWP has attracted increasing attention and is widely used for clinical treatment both in China and worldwide [15].

However, the therapeutic effect of TWP on pouchitis remains rarely reported. The purpose of this study was to investigate whether TWP ameliorates pouchitis in a DSS-model of rats and to determine its potential mechanism of action.

2. Materials and methods

2.1. Materials

Rabbit anti-occludin (bs-10011R) and rabbit anti-Zo-1 (PB0072) were purchased from BIOS (Beijing Bioengineering Company, China),

* Corresponding author.
E-mail address: landmark1503@sina.com (G. Liu).
[1] These authors contributed to the work equally and should be regarded as co-first authors.

http://dx.doi.org/10.1016/j.intimp.2016.12.008
1567-5769/

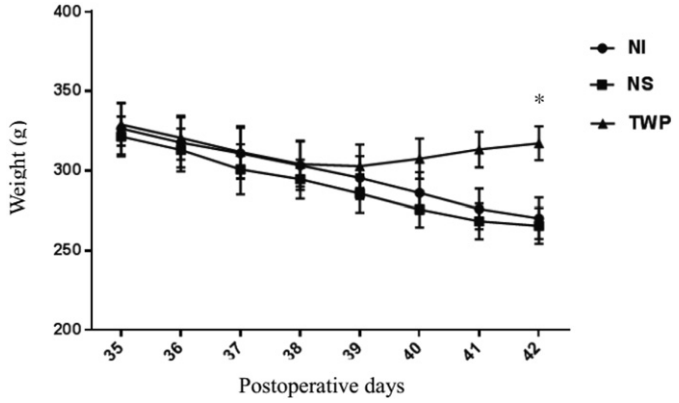

Fig. 1. Weight change in each group after intervention. At day 30 after IPAA, pouchitis was induced by drinking DSS for 4 days and then at day 35, rats were administrated with NI, NS, or TWP for 7 days. Weight loss was continuously decreased initially, but at day 39 the weight of rats in the TWP group began to increase. Data represent the means ± SD. $n = 6$ rats per group.

and SABC kit (SA1022), and DAB kit (AR1022) were purchased from Boster (Wuhan Boster Bioengineering Company, China). Dextran sulfate sodium was purchased from MP Biomedicals (molecular weight 36,000–50,000 MP Biomedicals, Soho, OH, USA).

2.2. Animals

Male Sprague-Dawley (Laboratory Animal Center of Military Medical Science Academy of the PLA in Chinese people's Liberation Army, China) rats weighing between 360 and 420 g were housed individually in cages (in a room controlled at 24 ± 2 °C with a relative humidity of 40–70% and a 12 h light/dark cycle) and fed standard rodent chow and fresh distilled water. Rats used in this study had comparable normal health status and were 10–12 weeks of age. All rats were free from specific pathogens and all animal experiments were performed according to international guidelines on animal research and ethics.

2.3. Induced pouchitis model and treatment protocols

Microsurgery was used to induce the IPAA model based on a standard protocol [16], and pouchitis was induced by administering 4% DSS to the drinking water for 4 consecutive days postoperation. Subsequently, the model rats were randomly divided into a no intervention (NI) group, normal saline (NS) group, and TWP group. Rats in the NS group were lavaged with normal saline at dose of 3 ml/day and those in the TWP group received a dose of TWP of 12 mg/kg/day for 7 days [17–19]. We evaluated the severity of pouchitis by the daily monitoring of clinical manifestations such as weight loss, diarrhea, and bloody stool.

2.4. General condition of animals and quality of stool

During the period of this study we carefully recorded the body weight, water, and diet intake of rats. The general condition of animals was also checked daily throughout the study including behavior, activity, hematochezia, and stool consistency. Fecal consistence and blood content were scored using a 5-point scale reported by Drzymala-Czyz [20]: (1 = lack of stool, 2 = diarrhea, 3 = blobby stool, 4 = textured stool, 5 = normal stool). The observer was 'blinded' to the rat group selection and the sample origin.

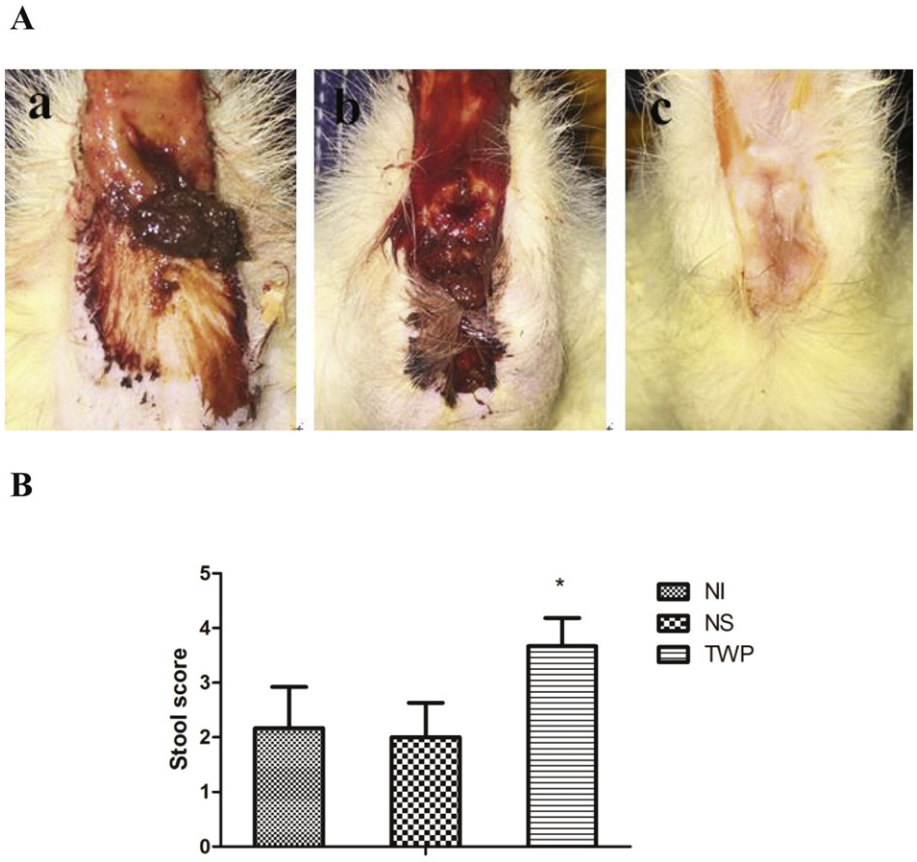

Fig. 2. Stools around the anus and the stool score at day 3 after NS or TWP intervention. (A) a, bloody stool around the anus of rats in the NI group; b, bloody stool on the anus of an NS group rat; c, the anus was clean and the bloody stools disappeared in the TWP group. (B) The feces score of rats in the TWP group (3.67 ± 0.516) was significantly higher than in the NI or NS group (2.17 ± 0.753, 2.00 ± 0.632) (*$P < 0.05$), and was similar in the NI and NS groups. Bars represent the means ± SD. $n = 6$ rats per group, $P > 0.05$.