Koisio Technology-Produced Water Significantly Decreased Inflammation and Multiple Injuries in Mouse Model of Dextran Sulfate Sodium Salt-Induced Acute Colon Inflammation

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Date: 2022-12-04T08:53:51+00:00

Abstract

Inflammation is one of the crucial pathological factors of numerous diseases. It is critical to search for new strategies to decrease inflammation-produced damage. Our previous study has reported that Koisio technology-produced cell culture media produced increased antioxidant capacity of cell cultures. Since oxidative stress plays a significant role in inflammation-produced tissue injury, in our current study we used a mouse model of acute colon inflammation to test our hypothesis that Koisio technology-produced water may decrease inflammation-produced tissue damage. Our study has obtained evidence supporting the hypothesis: First, Koisio technology-produced water significantly attenuated inflammation-induced shortening of colon length in the mouse model of Dextran Sulfate Sodium salt (DSS)-induced acute colon inflammation; second, Koisio technology-produced water significantly attenuated colon inflammation-induced increase in DAI in the mouse model; third, Koisio technology-produced water significantly attenuated colon inflammation-induced increase in the Spleen Index in the mouse model; and fourth, Koisio technology-produced water significantly attenuated the increases in the myeloperoxidase (MPO) activity and the Eosinophil peroxidase (EPO) activity in the mouse model. Collectively, our study has provided novel evidence suggesting that Koisio technology-produced water can decrease inflammation-induced tissue damage in the mouse model of acute colon inflammation.

Full Text

Preamble

Koisio Technology-Produced Water Significantly Decreased Inflammation and Multiple Injuries in Mouse Model of Dextran Sulfate Sodium Salt-Induced Acute Colon Inflammation

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Running Title: Koisio water decreases damage

Abstract

Inflammation represents a critical pathological factor in numerous diseases, making the search for novel strategies to mitigate inflammation-induced damage imperative. Our previous research demonstrated that Koisio technology-produced cell culture media significantly enhanced the antioxidant capacity of cell cultures. Given that oxidative stress plays a substantial role in inflammation-mediated tissue injury, we hypothesized that Koisio technology-produced water might reduce inflammation-induced tissue damage. Using a mouse model of acute colon inflammation, we obtained compelling evidence supporting this hypothesis. First, Koisio technology-produced water significantly attenuated inflammation-induced colon shortening in a Dextran Sulfate Sodium salt (DSS)-induced acute colitis model. Second, it markedly reduced the inflammation-associated increase in Disease Activity Index (DAI). Third, it significantly diminished the elevated Spleen Index resulting from colon inflammation. Fourth, it substantially suppressed the increased activities of myeloperoxidase (MPO) and eosinophil peroxidase (EPO). Collectively, our findings provide novel evidence that Koisio technology-produced water can ameliorate inflammation-induced tissue damage in a mouse model of acute colon inflammation.

Keywords: Inflammation; Colon damage; Colon length; Spleen index; Mouse model

Introduction

Inflammation constitutes a key pathological factor in numerous diseases, including stroke and cancer [1, 2]. Chronic inflammation also represents a major cause of cancer development [3-5]. Since long-term administration of anti-inflammatory drugs is costly and often produces toxic side effects, identifying novel, non-toxic, economical, and simple approaches to reduce inflammation-induced tissue injury is critical.

Our previous study indicated that Koisio technology-produced cell culture media significantly enhanced the antioxidant capacity of cell cultures [6]. Since a primary pathological mechanism of inflammation involves oxidative stress—for example, neutrophil myeloperoxidase (MPO) generates potent reactive oxygen species (ROS) [7, 8]—we hypothesized that Koisio technology-produced water might reduce inflammation-induced tissue injury. In this study, we report findings demonstrating that Koisio technology-produced water decreases both inflammation and inflammation-induced tissue injury in a mouse model of DSS-induced acute colon inflammation.

Materials and Methods

Materials

All chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise specified. Dextran Sulfate Sodium salt (MW: 36–50 kDa) was obtained from MP Biomedicals (Santa Ana, CA, USA). Male C57BL/6SLAC mice were purchased from SLRC Laboratory (Shanghai, China).

Methods

Animal Model of Acute Colon Inflammation

Male C57BL/6Slac mice weighing 18–24 g received either Koisio technology-produced water (prepared as previously described [6]) or regular water for 10 days. The experimental procedures followed established protocols [9]. Mice were then administered 3% (w:v) DSS (0216011080, MP Biomedicals) in either Koisio technology-produced water or deionized water for 7–10 days. Animals were monitored daily, with body weight and fluid consumption recorded. Fecal blood was detected using an occult blood test kit (C027-1-1, Nanjing Jiancheng Bioengineering Institute, China), with scoring criteria detailed in Table 1 . The Disease Activity Index (DAI) was calculated by summing daily scores across all parameters [10]. On the final experimental day, mice were euthanized and colons and spleens were harvested. Colon lengths were measured prior to washing and freezing, while spleens were stored in saline on ice for subsequent analysis.

Determination of Colon Length

Following euthanasia, colons were excised and stored in saline on ice. After collection, colons were arranged alongside a measuring scale on a white surface

and photographed. ImageJ software was used to measure colon lengths from the digital images.

Determination of Spleen Index
Whole spleens were harvested on the final day and stored in saline on ice. After collection, wet weights were measured using an analytical balance (Mettler Toledo AL104, Shanghai, China). The Spleen Index was calculated as spleen wet weight (mg) divided by final body weight (g) [11].

MPO Assay
MPO activity was measured as previously described [10, 12]. Colon tissue was weighed and placed in tubes containing 0.5% hexadecyltrimethylammonium bromide in 50 mM potassium phosphate buffer (4.35 g dibasic potassium phosphate and 3.4 g monobasic potassium phosphate per liter of deionized water, pH 6.0) at a ratio of 50 mg tissue per ml buffer. Samples were homogenized and centrifuged at 14,000 × g for 5 min at 4 °C. For the assay, 10 l of supernatant was combined with 200 l of o-dianisidine solution (0.167 mg/mL o-dianisidine dihydrochloride and 0.0006% hydrogen peroxide in 5 mM potassium phosphate buffer, pH 6.0). Absorbance changes at 460 nm were recorded at 30-second intervals for 5 minutes.

EPO Assay
EPO activity was measured as previously described [13, 14] using the same homogenization buffer as the MPO assay [15]. Colons were homogenized until no large tissue fragments remained, then centrifuged at 14,000 × g for 5 min at 4 °C. The reaction mixture contained 0.1 mM o-phenylenediamine dihydrochloride in 0.05 M Tris-HCl with 0.1% Triton X-100 and 1 mM hydrogen peroxide. Fifty l of supernatant was combined with 100 l of reaction solution and incubated at room temperature for 30 min, followed by addition of 50 l of 4 M sulfuric acid to terminate the reaction. Absorbance was measured at 492 nm.

Statistical Analysis
Data are presented as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls post hoc test. P values less than 0.05 were considered statistically significant.

Results

Koisio technology-produced water significantly attenuated DSS-induced colon shortening, DAI elevation, and Spleen Index increase in the mouse model of acute colon inflammation. DSS administration caused marked colon shortening, which was significantly ameliorated in mice that received Koisio technology-produced water (Fig. 1 [Figure 1: see original paper]). DSS also induced a substantial increase in DAI, which was significantly reduced in the Koisio water group (Fig. 2 [Figure 2: see original paper]). Additionally, DSS provoked a significant elevation in the Spleen Index that was notably suppressed by Koisio technology-produced water (Fig. 3 [Figure 3: see original paper]).

Furthermore, Koisio technology-produced water significantly attenuated DSS-induced increases in both MPO and EPO activities. DSS administration elevated MPO activity considerably, an effect that was significantly diminished in mice receiving Koisio technology-produced water (Fig. 4 [Figure 4: see original paper]). Similarly, DSS-induced EPO activity increases were significantly suppressed by Koisio water treatment (Fig. 5 [Figure 5: see original paper]).

Discussion

The major findings of our study are fourfold. First, Koisio technology-produced water significantly attenuated inflammation-induced colon shortening in the DSS-induced acute colitis mouse model. Second, it markedly reduced the inflammation-associated increase in DAI. Third, it significantly diminished the elevated Spleen Index resulting from colon inflammation. Fourth, it substantially suppressed the increased activities of MPO and EPO.

Our previous research demonstrated that cell cultures grown in Koisio technology-produced media exhibited significantly enhanced antioxidant capacity [6]. Since oxidative stress represents a key pathological mechanism of inflammation [7], we hypothesized that Koisio technology-produced water might reduce inflammation-induced tissue injury. Our current findings support this hypothesis, showing that Koisio technology-produced water decreased three key indices of tissue damage in the DSS-induced colitis model: colon shortening, increased Spleen Index, and elevated DAI.

Additionally, we found that Koisio technology-produced water significantly attenuated inflammation-induced increases in MPO and EPO activities. Since elevated MPO and EPO activities are important markers of inflammation, these results suggest that Koisio technology-produced water can reduce inflammation in this model. However, further investigation is warranted to elucidate the mechanisms underlying the inhibitory effects of Koisio technology-produced water on these enzyme activities.

Based on our previous finding that Koisio technology-produced cell culture media possess significant antioxidant capacity [6], we propose that Koisio technology-produced water reduces tissue damage in the DSS-induced colitis model partially by enhancing tissue antioxidant capacity. Our observation that Koisio technology-produced water significantly attenuated inflammation-induced increases in MPO and EPO activities also suggests important mechanisms underlying its protective effects. Future studies should further investigate the mechanisms through which Koisio technology-produced water ameliorates inflammation-induced tissue damage.

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Figure Legends

Figure 1. Koisio technology-produced water significantly attenuated colon inflammation-induced shortening of colon length in the mouse model of DSS-induced acute colon inflammation. Mice drank regular drinking water or Koisio technology-produced water for 10 days, then received 3% (w:v) Dextran Sulfate

Sodium salt (DSS) in deionized water or Koisio technology-produced water for 7–10 days. Colon lengths were then determined. ***, $P < 0.001$. N = 18–31.

Figure 2. Koisio technology-produced water significantly attenuated colon inflammation-induced increase in DAI in the mouse model of DSS-induced acute colon inflammation. Mice drank regular drinking water or Koisio technology-produced water for 10 days, then received 3% (w:v) Dextran Sulfate Sodium salt (DSS) in deionized water or Koisio technology-produced water for 7 days. DAI was then determined. ***, $P < 0.001$. N = 18–31.

Figure 3. Koisio technology-produced water significantly attenuated colon inflammation-induced increase in Spleen Index in the mouse model of DSS-induced acute colon inflammation. Mice drank regular drinking water or Koisio technology-produced water for 10 days, then received 3% (w:v) Dextran Sulfate Sodium salt (DSS) in deionized water or Koisio technology-produced water for 7–10 days. Spleen index was then determined. ***, $P < 0.001$. N = 18–31.

Figure 4. Koisio technology-produced water significantly attenuated the increases in MPO activity in the mouse model of DSS-induced acute colon inflammation. DSS induced significant increases in MPO activity, which was significantly attenuated in the mice that had drunk Koisio technology-produced water. ***, $P < 0.001$. N = 11–20.

Figure 5. Koisio technology-produced water significantly attenuated the increases in EPO activity in the mouse model of DSS-induced acute colon inflammation. DSS also induced significant increases in EPO activity, which was significantly attenuated in the mice that had drunk Koisio technology-produced water. ***, $P < 0.001$. N = 12.

Note: Figure translations are in progress. See original paper for figures.