Scientific Research Publishing

Health, 2023, 15, 938-943
https://www.scirp.org/journal/health
ISSN Online: 1949-5005
ISSN Print: 1949-4998

Effect of Baicalin on TNBS-Induced Colonic Inflammatory Injury in Rats

Yu Feng, Min Guo*

Department of Gastroenterology, The Fourth People's Hospital of Haikou, Haikou, China
Email: *g2002m@163.com

How to cite this paper: Feng, Y. and Guo, M. (2023) Effect of Baicalin on TNBS-Induced Colonic Inflammatory Injury in Rats. *Health*, **15**, 938-943.
https://doi.org/10.4236/health.2023.159062

Received: August 8, 2023
Accepted: September 1, 2023
Published: September 4, 2023

Abstract

Objective: The aim is to observe the protective effect of baicalin on trinitrobenzene sulfonic acid (TNBS)-induced experimental colitis in mice and explore its mechanism. **Methods:** The mice were divided into 4 groups: ethanol control group, TNBS model group, baicalin low-dose group and baicalin high-dose group. The model of experimental colitis in mice was induced by TNBS enema. After 2 hours of TNBS enema, baicalin was given by gavage, QD × 7D. The animals were sacrificed on the 8th day to observe the extent of colonic mucosal damage, and the Peroxidase activity, Malondialdehyde (MDA) and glutathione (GSH) contents were measured. **Results:** Compared with the TNBS model group, the body weight, gross injury score and histological changes were significantly improved; MPO enzyme activity and MDA content were significantly decreased in the low and high-dose baicalin groups; and the content of glutathione increased. **Conclusion:** Baicalin can alleviate TNBS-induced colitis in mice, and the mechanism is related to the antioxidation of baicalin.

Keywords

Baicalin, Trinitrobenzene Sulfonic Acid, Experimental Colitis, Mouse

1. Introduction

Scutellaria baicalensis, also known as *Camellia* root and Tujintea root, is a perennial herb belonging to the labiform family. In the middle period of Chinese medicine, *Scutellaria baicalensis* was classified into the heart, lung, gallbladder and intestine, and has the effects of clearing heat and dampness, purging fire and detoxicating. *Scutellaria baicalensis* is a commonly used traditional Chinese medicine. Its effective components are flavonoids, of which the highest content is *Scutellaria baicalin* and its aglytin. Studies have shown that baicalin has the function of

scavenging hydroxyl free radicals, superoxide free radicals and alkane free radicals, and plays a significant role in antioxidant and anti-inflammatory aspects [1]. Ulcerative colitis and Crohn's disease are non-specific chronic intestinal inflammatory diseases of unknown etiology, and recent studies have found that their pathogenesis may be closely related to oxygen free radicals and lipid peroxidation [2]. Therefore, we speculated that baicalin may have a certain protective effect on inflammatory bowel disease. In this experiment, we investigated the effects of Baicalin on the activity of Peroxidase acid (MPO) and the contents of Malondialdehyde (MDA) and glutathione (GSH) in the intestinal tissues of mice with experimental colitis, and Lipid peroxidation the effects of Baicalin on the levels of MDA and GSH in the intestinal tissues of mice with experimental colitis. In this study, the TNBS method was used to make an animal model of ulcerative colitis in recent years, to investigate the protective effect of Baicalin on trinitrobenzene sulfonic acid (TNBS)-induced experimental colitis in mice.

2. Materials

Baicalin (Nanjing Qingze Pharmaceutical Company, purity: 99.1%, batch number 050213), 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Sigma Company, batch number: SLBP0899V), sodium pentobarbital (Sigma Company, batch number: 20160516), 2,4,6-trinitrophenol (bitter acid) (Taishan Guangdong Overseas Chinese Reagent Plastics Co., Ltd., batch number: 20151001), MPO, MDA, GSH assay kit (Nanjing Jiancheng Institute of Biological Engineering), balb C mice, beth Rui (Hainan) Biotechnology Co., Ltd., Animal Certificate No. SYXK (Qiong) 2023-0030.

3. Methods

3.1. Animal Grouping and Model Establishment

Forty mice were randomly divided into 4 groups: ethanol control group, TNBS model group, baicalin low-dose group and baicalin high-dose group, with 10 mice in each group. After fasting for 24 hours, the mice were mildly anesthetized with ether and the catheter was carefully inserted into the colon. To induce experimental colitis in mice [3], 1 mg TNBS was dissolved in 50% ethanol to make 1%TNBS ethanol solution, and 0.1 mL of the solution was injected into the colonic cavity by catheter. The ethanol control group was injected by the same method Add 50% ethanol 0.1 mL. After the infusion, the mice were put back into the cage and weighed once a day.

3.2. Method of Administration

After 2 hours of TNBS infusion, baicalin low-dose group and high-dose group were given baicalin 30 $mg \cdot kg^{-1}$ and 60 $mg \cdot kg^{-1}$ by intragaxed administration, qd × 7, ethanol control group and TNBS model group were given equal volume of normal saline. On the 8th day, the animals were killed and the colonic tissues were taken to determine the following indexes.

3.3. Colitis Injury Degree Gross Shape Score

The mice were killed by decapitation, the colon was immediately dissected, the intestinal cavity was cut along the mesenteric margin, and the normal saline was rinsed. The inflammatory colon injury of the mice was observed by the naked eye. The scoring criteria were as follows: 0, no inflammation or ulcer was observed; 1 score, local congestion, no ulcer; 2 points, ulcer without congestion; 3 points, ulcers and inflammation in one site; 4 points, ulcers or inflammation in 2 or more sites; 5 points, ulcer length > 2 cm.

3.4. Colitis Histological Score

Colon tissues were taken, fixed with 10% formaldehyde solution, paraffin- embedded, sliced, and stained by HE. The inflammatory colon injury of mice was observed under a microscope, and the scoring system was as follows. 0 score, no inflammation; 1, there is very little leukocyte infiltration; 2 points, a small amount of leukocyte infiltration; 3 points, more white blood cell infiltration, increased blood vessel density, intestinal wall thickening; 4 minutes, the whole layer of the colon has leukocyte infiltration, goblet cell loss, increased blood vessel density, and intestinal wall thickening [4].

3.5. Colon MPO, MDA and GSH Measurements

After the mice were killed, the colon tissue was prepared into 10% homogenate, and the supernatant was taken. The activity of the MPO enzyme and the content of MDA and GSH were determined according to the kit instructions.

3.6. Statistical Methods

Analysis of variance (ANOVA) was used to compare the groups, and single factor analysis of variance (ANOVA) was used to compare the groups. $p < 0.05$ indicated that the difference was statistically significant, and $p < 0.01$ indicated that the difference was significant, $p < 0.001$ indicated that the difference was very significant.

4. Results

4.1. Effects of Baicalin on Body Mass of TNBS Induced Experimental Colitis Mice

After the administration of TNBS, the body mass of mice in the TNBS model group was gradually reduced, and by the end of the experiment, the body mass of mice in the TNBS model group was reduced by 3.49 g on average, which was significantly different from that in the ethanol control group ($p < 0.01$) (Table 1). And give 30 $mg \cdot kg^{-1}$

4.2. Effects of Baicalin on Inflammatory Injury of Colon Tissue in Mice

As can be seen from Table 2, both the gross damage score and histological damage

score of mice in TNBS model group were significantly higher than those in ethanol control group ($p < 0.01$), indicating that colon tissue of mice had obvious inflammatory damage. After administration of 30 mg·kg^{-1} and 60 mg·kg^{-1} baicalin, both the gross and histological damage scores of mice were decreased, and the difference was very significant compared with TNBS model group ($p < 0.01$), indicating that baicalin could alleviate TNBS-induced colon tissue inflammatory injury.

4.3. Effects of Baicalin on MPO Activity and MDA and GSH Content in Colon Tissue

After the TNBS enema, MPO activity and MDA content in the colon tissue of the TNBS model group were significantly increased compared with the ethanol control group, while GSH content was significantly decreased ($p < 0.01$). Administration of 40 mg·kg^{-1} and 80 mg·kg^{-1} baicalin significantly decreased the MPO activity and MDA content in the colon tissue of TNBS-induced mice, and significantly increased the decrease of GSH content in the colon tissue of TNBS-induced mice, which was significantly different from TNBS model group Significant ($p < 0.01$). See Table 3.

Table 1. Effect of baicalin on body weight in mice ($\bar{x} \pm s$).

Group	Dose/mg·kg^{-1}	Before administration	After administration	Body mass change
Ethanol control group	-	18.6 ± 1.01	20.2 ± 1.03	15 ± 0.20
TNBS model group	-	18.8 ± 1.10	15.5 ± 1.00	3.2 ± 1.11*.
Baicalin lowdose group	30	18.9 ± 1.00	17.6 ± 0.94	1.4 ± 0.34**
Baicalin high-dose	60	19.1 ± 1.01	18.7 ± 0.70	0.5 ± 0.12**

Note: Compared with ethanol control group, *$p < 0.01$; Compared with TNBS model group, **$p < 0.01$.

Table 2. Effect of baicalin on experimental colitis injury in mice ($\bar{x} \pm s$).

Group	Dose/mg·kg^{-1}	Gross injury score	Histological injury score
Ethanol control group	-	0.31 ± 0.15	0.5 ± 0.12
Ethanol control group	-	3.5 ± 0.12*	3.1 ± 0.76*
Ethanol control group	30	2.6 ± 0.12**	2.3 ± 0.26**
Ethanol control group	60	1.3 ± 0.11**	1.3 ± 0.21**

Note: Compared with ethanol control group, *$p < 0.01$; Compared with TNBS model group, **$p < 0.01$.

Table 3. Effects of baicalin on MPO activity and contents of MDA and GSH of colon in mice ($\bar{x} \pm s$).

Group	Dose/mg·kg^{-1}	MPO/U·g^{-1}	MDA/nmol·g^{-1}	GSH/μg·g^{-1}
Ethanol control group	-	1.1 ± 3.17	6.9 ± 2.81	174.1 ± 22.41
Ethanol control group	-	44.1 ± 8.11*	17.1 ± 2.94*	58.1 ± 24.02*
Ethanol control group	30	25.1 ± 6.90**	12.0 ± 0.07**	111.2 ± 28.4**
Ethanol control group	60	17.1 ± 7.01**	8.6 ± 1.09**	135.5 ± 34.2**

Note: Compared with ethanol control group,*$p < 0.01$; Compared with TNBS model group, **$p < 0.01$.

5. Discussion

When the body is formed by excess oxygen free radicals or the ability of the antioxidant system is weakened, in addition to the direct damage of tissue cells by oxygen free radicals, it can also trigger the chain reaction of polychain unsaturated fatty acids on the cell membrane, and produce lipid peroxidation products such as MDA [5]. Lipid peroxidation reaction and the resulting end products such as MDA can lead to DNA damage, protein denaturation and crosslinking, loss of enzyme activity, and loss of enzyme activity. The Lipid peroxidation reaction and its end products, such as MDA, can lead to DNA damage, denaturation and cross-linking, loss of enzyme activity, damage of cell membrane structure and release of inflammatory mediators [6]. In this study, experimental colitis in mice was induced by TNBS, and the results showed that the body mass of mice was reduced, the content of MDA, the end product of lipid peroxidation was increased, the content of endogenous antioxidant GSH was decreased, and ulcers and inflammatory cell infiltration were observed by naked eye and microscope, and the inflammatory damage was obvious, which was consistent with literature reports. Baicalin is a kind of flavonoid compound extracted from the dried root of *Scutellaria baicalensis* Georgi, a plant in the labiaceae family, and is one of the main effective components in *scutellaria baicalensis* [7] [8]. In this experiment, after the treatment of mice with high and low doses of baicalin, the increase of MDA content in colon of TNBS-induced mice can be significantly reduced, the decrease of GSH content in colon of TNBS-induced mice can be restored, and the inflammatory damage of colon of mice can be alleviated, indicating that baicalin can alleviate the damage of colon lipid peroxidation induced by TNBS and reduce the consumption of endogenous antioxidant GSH. MPO is an enzyme with a high content of neutrophils, and the detection of MPO can better reflect the neutrophils infiltration during the acute inflammatory period (within 1 week after inflammation) and is one of the important indicators to evaluate the degree of inflammatory injury [9] [10] [11].

6. Conclusion

The results of this experiment showed that the MPO activity of mice in the TNBS model group was significantly increased, while the colon MPO activity of mice treated with high and low doses of baicalin was significantly decreased compared with the TNBS model group, indicating that baicalin could significantly alleviate TNBS-induced colon inflammatory injury. The results of this study confirm that baicalin can effectively reduce colon inflammatory response, which may be related to its scavenging of oxygen free radicals and antioxidant effects, suggesting that baicalin can be developed as a potential anti-inflammatory bowel disease drug.

Fund Project

2022 Hainan Provincial Key Research and Development Program, project number: ZDYF2022SHFZ117.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Shen, H., Tang, Z.P., Tang, X.D., *et al.* (2019) Traditional Chinese Medicine Diagnosis and Treatment Guide for Ulcerative Colitis, A Common Disease of Digestive System. *Chinese Journal of Traditional Chinese Medicine*, **34**, 4155-4160.

[2] Kakuta, Y., Ichikawa, R., Fuyuno, Y., *et al.* (2020) An Integrated Genomic and Transcriptomic Analysis Reveals Candidates of Susceptibility Genes for Crohn's Disease in Japanese Populations. *Scientific Reports*, **10**, Article No. 10236. https://doi.org/10.1038/s41598-020-66951-5

[3] Ungaro, R., Mehandru, S., Allen, P.B., *et al.* (2017) Ulcerative Colitis. *Lancet*, **389**, 1756-1770. https://doi.org/10.1016/S0140-6736(16)32126-2

[4] Noureddine, O., Gatfaoui, S., Brandan, S.A., *et al.* (2020) Experimental and DFT Studies on the Molecular Structure, Spectroscopic Properties, And Molecular Docking of 4-Phenylpiperazine-1-Ium Dihydrogen Phosphate. *Journal of Molecular Structure*, **1207**, Article 127762. https://doi.org/10.1016/j.molstruc.2020.127762

[5] Li, Y.J., Liu, Y.X., Tian, W.C., *et al.* (2018) Studies on the Geographical Variation of the Contents of Medicinal Components in Scutellaria Baicalensis Georgi from Different Provenances. *Chinese Journal of Traditional Chinese Medicine*, **43**, 234-236.

[6] Sun, P.L., Zhong, M., Li Y.B., *et al.* (2016) Research Progress on Etiology and Pathogenesis of Ulcerative Colitis in Chinese Medicine. *Chinese Medicine Research*, **29**, 71-74.

[7] Yuan, C.Y., Liu, B.T., Huang, J.Y., *et al.* (2019) Application of Network Pharmacology in Screening and Mechanism of Action of Chinese Medicine. *Guangzhou Chemical Industry*, **47**, 20-22.

[8] Meng, F.C. and Tang, L.D. (2019) Problems and Prospects in Network Pharmacology of Chinese Medicine. *Chinese Herbal Medicine*, **51**, 2232-2237.

[9] Ren, Y.L. and Jia, X.H. (2020) Thinking on Clinical Diagnosis of Ulcerative Colitis. *Zhejiang Journal of Traditional Chinese Medicine*, **55**, 918-919.

[10] Xing, J.F., Hou, J.Y., Zou, J.M., *et al.* (2005) Effect of Kuijiekang Capsule on TNBS Induced Colitis in Rats. *Chinese Materia Medica*, **28**, 315-318.

[11] Zhen, J.H. and Huang, G.R. (2019) Comparison and Review of Traditional Chinese Medicine Diseases, Etiology and Pathogenesis of Ulcerative Colitis. *Global Journal of Chinese Medicine*, **12**, 1286-1289.