Protection of Gastrointestinal Mucosa from Acute Heavy Alcohol Consumption: The Effect of Berberine and Its Correlation with TLR2, 4/IL1β-TNFα Signaling

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Abstract

The purpose of the present study is to confirm the protective effect of berberine (BBR) on gastrointestinal injury caused by acute heavy alcohol exposure, an effect that has not been reported previously. Our research details how BBR protects against gastrointestinal injuries from acute alcohol exposure using both in vivo and in vitro experiments. Acute high alcohol concentrations lead to obvious damage to the gastrointestinal mucosa, resulting in necrosis of the intestinal mucosa. Oral administration of BBR was able to significantly reduce this alcohol-induced damage, inhibit increases of alcohol-induced TNFα and IL-1β expression in gastrointestinal mucosa as well as their upstream signals TLR2 and TLR4, and regulate cytokines that modulate tight junctions. Alcohol consumption is a popular human social behavior worldwide, and the present study reports a comprehensive mechanism by which BBR protects against gastrointestinal injuries from alcohol stress, providing people with a novel application of BBR.

Introduction

The Chinese medical herb, Coptidis Rhizoma, has a long history of clinic use and use as a food supplement. In ancient Chinese medical literature, Coptidis Rhizoma was originally recorded for its applications in the treatment of gastrointestinal dysfunction, including diarrhea, dysentery, and inflammation. Berberine (BBR) is the most abundant active natural compound in Coptidis Rhizoma and plays a major role in the pharmacological effects of Coptidis Rhizoma, including anti-cancer, anti-stroke, anti-diabetes and anti-hyperlipidemia effects [1–7]. BBR was recently reported to exhibit novel anti-inflammatory and anti-oxidant properties as well as...
the ability to inhibit gene transcription [8–11]. Recent studies reported the ability of BBR to maintain the junctions between intestinal mucosa [12–14], and the efflux pump in the small intestine mucosa can promote highly regional distribution of BBR in the gastrointestinal epithelia [15–19]. These findings indicate a potential application of BBR in the prohibition of gastrointestinal injury by excessive alcohol use. Alcohol consumption is a common social behavior worldwide. Alcohol use is a part of the culture and daily life of more than 2 billion people worldwide [20], and can play an essential role in business and social activities. After alcohol drinking, especially acute and extensive consumption, alcohol stimulates the gastrointestinal tract and causes stress to the gastrointestinal mucosa, inducing gastrointestinal bleeding, inflammatory damage, and ulcers due to the upregulation of pro-inflammatory cytokines, IL-1β, IL-6 and TNFα [21–23].

Based on prior studies, we hypothesized and tested the potential application of BBR in protecting against alcohol stress-induced gastrointestinal injuries. Both in vivo and in vitro experimental models of acute alcohol exposure were used to evaluate the effect of BBR on alcohol injury. The pro-inflammatory cytokines TNFα and IL-1β and the TLR2, TLR4, and NOD2 innate immunity signaling pathways were determined to be dynamically involved in gastrointestinal alcohol injury. BBR could effectively antagonize the regulation of the proinflammatory cytokine profile and alter innate immunity signaling downstream of acute extensive alcohol stress through direct effects on gene transcription. Furthermore, BBR antagonized elevations of blood alcohol by prohibiting alcohol absorption and up-regulating ADH (alcohol dehydrogenase) activity to accelerate the metabolism of absorbed alcohol.

Methods and Materials

Experimental animals, drugs and chemicals

Male ICR mice weighing 18–22 g were purchased from Vital River Laboratories (Beijing, China). The animals were housed in temperature- and humidity-controlled rooms, kept on a 12 h light/dark cycle and provided with unrestricted amounts of rodent chow and drinkable water. The laboratory animal facility was accredited by the AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International), and the IACUC (Institutional Animal Care and Use Committee) of Tsinghua University approved all animal protocols used in this study (Approval ID: 13-DLJ1).

Berberine hydrochloride was purchased from the Beijing Shuanghe Pharmacy Co. Ltd. (Batch No. 110905) (Beijing, China). Berberine hydrochloride standards were purchased from the National Institutes for Food and Drug Control (Beijing, China) (Batch No. 110713–200609). Isolated alcohol (analytical grade) was purchased from Beijing Chemical Plant (Beijing, China).

Dosage and use of BBR and alcohol

Based on our prior experiments, BBR was administered orally at 75,150, or 300 mg/kg in saline vehicle. Three hundred milligrams/kilogram was far from the toxic dosage, and 150 mg/kg is the equivalent dose of adults in clinic [15].

Sixty percent alcohol (V/V) was employed for the experiments. Alcohol and normal saline was mixed to a final concentration of 60%. Based on the preliminary experiments, the volume for oral administration was 15 ml/kg (approximately 7.103 g/kg), and the samples were collected 2 hours after alcohol oral administration. BBR was administered orally 1 hour prior to alcohol consumption.
Experimental procedures in vivo

**Absorption of alcohol and enzymatic activity.** ICR mice were divided into five groups randomly (six mice in each group). BBR was administered orally to animals in three different groups. One hour later, 60% alcohol (0.15 ml/10 g body weight) was administered orally. The normal group was administered saline only, and the model group was administered alcohol only. After two hours, blood samples were collected through the supraorbital veins, and the serum was isolated by centrifugation (15000 rcf/g, 10min, 4°C). All samples were stored at -80°C for alcohol concentration and enzyme activity determination.

**Histopathological examination and diagnosis of gastrointestinal mucosa.** The mice were randomly separated into five groups as previously described. Each group was comprised of six mice. Two hours after the administration of alcohol, the mice were sacrificed for morphological examination. All stomach and intestinal tissues were separated for gene expression analysis and histological determination. The histopathological diagnoses were performed through hematoxylin-eosin (HE) staining by two different research scientists independently. The samples were semi-quantitatively scored according to Shackelford et al [24].

**Blood alcohol concentration.** Alcohol concentrations were measured in accordance with the reference values. The reaction system was as follows: 1.5M Tris-HCl buffer (pH 8.8) 350 μl, NAD⁺ (10 mg/ml) 100 μl, ADH (200 U/ml) 10 μl and serum samples 1 μl. The samples were mixed, and the OD value was recorded using a biochemical analyzer (Biosine Bio-technology and science Inc., China). The tested wavelength was set at 340 nm. The alcohol concentration is expressed in mg/ml.

**ADH activity determination.** ADH activity was measured in accordance with the instructions of the ADH kit (Nanjing Jiancheng Bioengineering Institute, China). The OD value was recorded using a microplate reader (BioRad, Model 680, USA) at OD₄₅₀nm. The enzyme activity is expressed in U/ml.

Experimental procedures in vitro

**Cell culture and cell viability assay.** The Caco2 and 293T cell lines were obtained from the Cell Culture Center of Chinese Academy of Medical Science (Beijing, China) and maintained at 37°C in a humidified incubator containing 5% CO₂. The cytotoxicity of BBR and alcohol to Caco2 cells and 293T cells was evaluated by MTT assay performed according to the method described in reference [25].

**Alcohol-induced responses in the Caco2 cell line.** 2 μg/ml BBR (saline used as vehicle) was added one hour before acute alcohol exposure, and the concentration was maintained throughout the experiment. Two hours after alcohol administration, protein and RNA samples were extracted.

**Promoter-GFP plasmid construction and transfection.** The TLR2-pEGFPN1, NOD2-pEGFPN1 and pEGFP-N1 plasmids were kindly provided by Dr. Xiao-Jin Yan and Professor Ye-Guang Chen, School of Life Science, Tsinghua University. The TLR4 gene promoter was obtained from the mouse genome by PCR. Mouse genomic DNA was extracted from mouse liver homogenate (TIANamp Genomic DNA Kit, Tiangen Biotech, China). The CMV promoter of the pEGFP-N1 plasmid was replaced by the TLR4 promoter. GFP expression driven by the TLR2, TLR4 or NOD2 promoter was determined after transfection into the 293T cell line. The primer sequences of the TLR4 promoter were as follows: sense: 5’- AGAACAATGAGGGACCCAGTCCGTTCCCTGGTTG -3’ and antisense: 5’- GGGATTCAAGCTTCCTGGTGTG -3’, generating a 1835-bp DNA fragment. The primer sequences of GFP were as follows: 5’- GCAGAAGAACAGGATCGGTTGCTCAGGGTAG -3’ and antisense: 5’- CGGACTGGGTGCTCAGGGTAG -3’.
Real time PCR and western blot

mRNA and protein determination was performed using q-PCR and western blot (WB) assay as described in detail previously [26]. For real-time PCR, all primer sequences were designed by NCBI GenBank and produced by Sangon Biotechnology Ltd. (Shanghai, China) (S1 Table and S2 Table). For western blot analysis, primary antibodies against NOD2 (rabbit monoclonal antibody [EPR16252], ab197030), TNFα (mouse monoclonal antibody [52B83], ab1793), IL-1β (rabbit polyclonal antibody, ab9722), TLR2 (mouse monoclonal antibody [T2.5], ab16894), TLR4 (mouse monoclonal antibody [76B357.1], ab22048), Occludin (rabbit polyclonal antibody, ab64482) and claudin4 (rabbit polyclonal antibody, ab15104) were purchased from Abcam (UK). Secondary antibodies of goat anti-mouse IgG-HRP (sc-2005) and goat anti-rabbit (sc-2004) IgG-HRP were purchased from Santa Cruz (USA). The targeted proteins were and visualized with the Super Signal West Femto Chemiluminescent Substrate (Thermo scientific pierce) and the intensity of visualized bands were analyzed by using Quantity One software (Bio-rad). β-actin was used as an internal control. Data were expressed by the ratio to β-actin.

Data analysis

Data are expressed as the mean ± S.D. Data were statistically analyzed using Kruskal–Wallis test. The test was performed using R software (USA). The non parametric Mann-Whitney U Test between two groups was performed after Kruskal–Wallis test. P values below 0.05 were considered statistically significant.

Results

Mouse gastrointestinal mucosal morphology and plasma alcohol concentration and ADH activity

Two hours after 60% alcohol administration, the alcohol-induced pathological damage was observed as a congestive and dark red appearance in the duodenum compared with that of normal mice. High-dose BBR (300mg/kg) effectively inhibited the alcohol-induced morphological changes of the duodenum (Fig 1A). By light microscopy, congestion, edema, necrosis and shedding of the mucosa from duodenum was observed in alcohol-treated mice (Fig 1B and 1D). BBR was able to effectively antagonize the alcohol-induced pathological changes in the duodenum, which was indistinguishable in morphology from the saline group. Unlike the small intestine, the gastric mucosa exhibited minor pathological changes among the different groups (Fig 1C). It has previously been reported that 100% alcohol can cause erosion in rat stomach mucosa and can up-regulate the mRNA expression of c-fos, c-jun and HSP70 in the damaged epithelium; however, the necrosis in the stomach was less severe than in the small intestine [27], suggesting that the small intestines were more sensitive to alcohol damage.

The blood alcohol concentration (BAC) after alcohol consumption is the major factor causing drunkenness and body damage. Pretreatment with BBR before alcohol administration significantly reduced the BAC in mice even at the lower doses (Fig 2A). Furthermore, the plasma ADH activity was remarkably increased in the group treated with high-dose BBR, suggesting that in addition to the protective duodenum effect, BBR could protect mice from alcohol injury through decreased blood alcohol concentration and enhanced metabolism of alcohol by increased ADH activity (Fig 2B).
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Fig 1. General observation and morphology of gastro-intestines after acute alcohol exposure (H.E. staining). Berberine (BBR) was administered at three different doses of 75, 150 and 300 mg/kg. A 60% alcohol was employed. The alcohol was administered at dose of 0.15 ml/10g body weight. The arrows indicate congestive necrosis places. (A): Observation of stomach and small intestines. Intestinal congestion occurs in the duodenum in model mouse. (B): Morphology of small intestines after alcohol administration. In the mouse model, small intestinal mucosa appears necrosis. BBR could prevent alcohol injury from the intestines. (C): Morphology of stomach after alcohol exposure. (D): Mucosa of small intestines (magnified 200 times). Alcohol causes gastric mucosal injury, edema with light staining. (E): Statistical score of the histopathological diagnoses for small intestines injury after alcohol consumption. Kruskal-Wallis chi-squared = 24.0696, df = 4, P = 7.735e-05. Data are expressed as the mean ± S.D. from six different mice. ###, vs. normal mice, P < 0.001. **, vs. model mice, P < 0.01.

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Effect of BBR on the pro-inflammatory cytokines profile and pattern recognition receptors in mouse stomach after acute alcohol exposure

To understand the inflammatory response and abnormal expression of pattern recognition receptors (e.g., Toll-like receptors (TLRs) and NOD2) accompanying the ethanol-induced gastrointestinal mucosal injury, we studied the expression of TLR2, TLR4 and NOD2 and their down-stream effectors TNF\(\alpha\) and IL1-\(\beta\) in mouse stomachs after acute alcohol exposure by q-PCR and western blot. The results revealed that acute alcohol exposure could significantly up-regulate the transcription and protein level of TNF\(\alpha\), IL1-\(\beta\), TLR2, and TLR4, and these alcohol-dependent enhancements were antagonized by BBR administration (Fig 3). Only one BBR dose group exhibited decreased expression of NOD2 protein as well as consistent q-PCR results, suggesting that BBR exhibits a greater effect on TLR2 and TLR4 compared with NOD2 in the stomach mucosa.

Occludin and claudin4 are major components of tight conjunctions in the gastrointestinal epithelium and act to regulate intestinal epithelial permeability. Acute high alcohol exposure can up-regulate occludin and claudin4 expression. However, alcohol-induced

![Graph A: Alcohol concentration](image1)

**Fig 2. The concentration of alcohol and activity of ADH enzyme in blood of mice.** (A): Alcohol, Kruskal-Wallis chi-squared = 26.6366, df = 4, \(P = 2.354e-05\). (B): ADH. Kruskal-Wallis chi-squared = 14.0461, df = 4, \(P = 0.007149\). Data are expressed as the mean \(\pm\) S.D. from six different mice. #, ## vs. normal mice, \(P < 0.05\), \(P < 0.01\). *, ** vs. model mice, \(P < 0.05\), \(P < 0.01\). doi:10.1371/journal.pone.0134044.g002
Fig 3. The expressions of inflammatory cytokines of mouse stomach after acute alcohol exposure. The concentration of alcohol was 60%. (A–G): mRNA expressions using real time PCR assay. (H–N): The expressions of protein using western blot assay. Berberine (BBR) was administered at three different doses of 75, 150 and 300 mg/kg. Data are expressed as the mean ± S.D. from six different mice. ## vs. normal mice, P < 0.01, * vs. model mice, P < 0.05, P < 0.01.

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Expression profile changes could be antagonized by BBR, which attenuated occludin and claudin4 expression (Fig 3F and 3G & 3M and 3N).

Effect of BBR on the pro-inflammatory cytokines profile and TLRs and NOD2 in mouse small intestine after acute alcohol exposure

Consistent with the studies in stomach, acute alcohol administration significantly increased the expressions of TNFα, IL1-β, TLR2, and TLR4 at the mRNA and protein level (Fig 4), and BBR dose-dependently inhibited alcohol-induced changes. However, inconsistent with NOD2 expression in the stomach, BBR inhibited the mRNA and protein upregulation of NOD2 caused by alcohol (Fig 4C& 4J), suggesting the greater sensitivity of the NOD2 response of the small intestinal mucosa to alcohol stress. The expression of other proteins was consistent with the mRNA results. In the small intestine, alcohol administration suppressed the expression of occludin but increased the expression of claudin4. BBR enhanced occludin expression and attenuated claudin4 expression (Fig 4F and 4G & 4M and 4N).

Effect of BBR and alcohol on the Caco2 cell line in vitro after alcohol exposure

To verify the effect of alcohol on small intestinal mucosal injury, we performed in vitro experiments using Caco2 cells, a human colon adenocarcinoma line exhibiting differentiation of small intestine epithelial cells [28]. Alcohol up-regulated the mRNA expression of the pro-inflammatory cytokines IL-1β and TNFα as well as the expression of the innate immune receptors TLR2, TLR4 and NOD2, which is consistent with the results observed in mice. BBR effectively decreased the expression of these genes, with the exception of NOD2 (Fig 5A–5E). In Caco2 cells, BBR exhibited little effect on NOD2 (Fig 5C). Similar results were observed at the level of protein expression (Fig 5F–5J). BBR down-regulated the protein expression of TLR2, but the trend did not reach statistical significance (Fig 5F). A 2% concentration of alcohol was administered (348 mmol/L) because the safety dosage for alcohol cytotoxicity was determined to be 696 mmol/L using the MTT assay (Fig 5K). BBR was administered at a dose of 2 μg/ml (5.95 μmol/L) in the experiments, which is far lower than the cytotoxic dosage of 37.2 μmol/L. The cytotoxicity of alcohol and BBR in Caco2 cells was measured using MTT assay (Fig 5L).

Effect of BBR on TLR2, TLR4 and NOD2 promoters in vitro

To acquire a more thorough understanding of the effect of BBR on TLR2, TLR4 and NOD2 expression, three promoter-driven expression plasmids were constructed (Fig 6A). Instead of CMV promoter, the TLR2, TLR4 or NOD2 promoters were used to drive the expression of green fluorescent protein (GFP). The expression of GFP was detected using q-PCR and WB assays. Alcohol promoted the mRNA expression of GFP downstream of the TLR2, TLR4 and NOD2 promoters. BBR was able to attenuate these stimulations and suppressed the up-regulated GFP mRNA expression driven by the TLR2 and TLR4 promoters (Fig 6B and 6C). However, BBR was unable to down-regulate GFP mRNA expression driven by the NOD2 promoter (Fig 6D). The protein expression of GFP in the TLR2 and TLR4 promoter plasmids were also inhibited by BBR, consistent with the results of mRNA expression (Fig 6E and 6F). Although the GFP protein expression of the NOD2 promoter plasmid exhibited a trend of down-regulation, it failed to reach statistical significance (P = 0.079) (Fig 6G). Therefore, we suggest that BBR suppresses alcohol-induced TLR2/TLR4 expression by interaction with their promoters. In these experiments, cells were treated with 44 mmol/L alcohol and 0.5 μg/ml (1.49 μmol /L)
Fig 4. The expressions of inflammatory cytokines of mouse small intestines after acute alcohol exposure. The concentration of alcohol was 60%. (A–G): mRNA expressions using real time PCR assay. (H–N): The expressions of protein using western blot assay. Berberine (BBR) was administered at three different doses of 75, 150 and 300 mg/kg. Data are expressed as the mean ± S.D. from six different mice. ## vs. normal mice, *P < 0.05, **P < 0.01.

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Fig 5. Expressions of inflammatory cytokines after berberine (BBR) on Caco2 cells injured by alcohol exposure in vitro. (A–E): mRNA expressions using real time PCR assay. (F–J): The expressions of protein using western blot assay. Alcohol was used at concentration of 348 mmol/L. BBR was administered at concentration of 5.95 μmol/L. (K): Cellular viability after alcohol exposure. (L): Cellular viability after BBR administration. Data are expressed as the mean ± S.D. from six independent experiments. #, ## vs. the control, *P < 0.05, **P < 0.01. ** vs. the model, *P < 0.01.

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BBR, based on the results of a safety dose of alcohol of less than 87 mmol/L and a safety dose of BBR of less than 2.94 μmol/L in 293T cells (Fig 6I and 6J).
Discussion

Alcoholic beverages have been consumed worldwide since the very beginning of recorded history [29]. Long-term or extensive drinking can cause harmful stresses to the central nervous system (CNS), cardiovascular system, immune system, liver, pancreas, gastrointestinal system, etc. [30, 31]. Thus, developing a strategy to alleviate alcohol consumption-related injury is pursued by many research groups worldwide.
In our preliminary experiments, alcohol induced the upregulation of inflammatory cytokines and the innate immunity response receptors TLR2, TLR4, and NOD2 in a dose-dependent manner and sixty percent alcohol could significantly alter the molecular profiles *in vivo* (Figures A and B in S1 File). Sixty percent alcohol-containing beverages are widely available on the market and are popular among alcohol consumers. Thus, 60% alcohol was selected to study the protective effect of BBR on alcohol-induced injury of the gastrointestinal system.

In some cases, extensive alcohol consumption is followed by inflammation [32, 33]. Alcohol-mediated inflammation signals are caused by the increased production of pro-inflammatory cytokines. In the present research, both *in vivo* and *in vitro* experiments revealed that alcohol could significantly promote the expression of the pro-inflammatory cytokine TNFα and IL1-β, which subsequently evokes inflammation. BBR antagonized alcohol-induced inflammation via suppressing the expression of pro-inflammatory cytokines.

Pattern recognition receptors act as the signaling molecules upstream of pro-inflammatory cytokines, such as IL-1β and TNFα [34–36]. The TLR4/MyD88 pathway had been confirmed as a target of alcohol-induced brain injury [37, 38]. TLR2 is an important mediator of inflammation in the airway epithelium induced by acute alcohol consumption [39]. The present results revealed that alcohol could induce TLR2, TLR4 and NOD2 upregulation, and pre-treatment with BBR was able to antagonize the alcohol-enhanced expression of TLR2 and TLR4. Experimental results from recombinant plasmids studies indicated that gene transcription initiation is the direct target of BBR with respect to its antagonistic role on alcohol-regulated gene expression profiles. However, the antagonistic effect of BBR on alcohol-induced NOD2 signal pathway alternations was not obvious, indicating that the involvement of the NOD2 pathway in alcohol-induced inflammation differs somewhat from TLR2 and TLR4 and remains to be elucidated.

Occludin and claudins are the main proteins responsible for the gastrointestinal tract epithelial tight junctions (TJs) and the regulation of intestinal epithelial permeability [40, 41]. Occludin knockout mice exhibit decreased density and poor organization of the tight junctions in the intestinal mucosa as well as functional defects [42, 43]. The claudin super family plays critical roles in barrier formation and the selective permeability in tissues [44–48]. In the present study, acute heavy alcohol consumption resulted in an abnormal expression profile of occludin and claudin4 in the gastrointestinal mucosa, and BBR antagonizes these profile changes. Interestingly, we observed that alcohol could stimulate occludin downregulation and claudin4 upregulation in the small intestines, which has been previously reported [49]. BBR was able to down-regulate claudin4 and up-regulate occludin, returning levels to homeostasis, suggesting that BBR’s potential effect on intestinal permeability altered alcohol-induced damage. Taken together, our results showed that alcohol could not only lead to an inflammatory reaction, but also affect mucosal permeability by modulating occludin and claudin4 expression. It was reported that TLR2 could influence the tight conjunction barrier in epidermal keratinocytes or cerebral endothelial cells [50–51]. BBR could inhibit the expression of TLR2, which would be involved in the mechanism of BBR on claudin4 and occludin. These putative mechanisms warrant further study.

Studies have demonstrated that inflammatory disorders induced elevated levels of IL-1β protein, followed by inhibition of the expression of occludin mRNA and enhanced intestinal mucosal permeability of TJs [52–54]. According to these reports, high alcohol consumption can promote intestinal permeability [55]. The present results revealed that acute high alcohol intake could cause injury of the mucosal layer of mouse small intestines, which was associated with up-regulated IL-1β expression and occludin suppression. BBR exhibited a consistent inhibitory effect on IL-1β expression, correlating with occludin expression up-regulation. TNFα is believed to be important in the incidence and development of inflammatory intestinal disease.
Targeting TNFα can inhibit intestinal inflammation and improve intestinal permeability [56]. Our data demonstrates that high alcohol intake can increase the expression of TNFα in the duodenum and that BBR can reduce the upregulation of TNFα, suggesting that inhibition of TNFα expression is involved in the effects of BBR on acute heavy alcohol consumption-induced damages.

European men are reported to consume more than 0.72 g/kg alcohol daily, and women consume more than 0.65 g/kg [57]. On average, the daily normal alcohol consumption among Europeans is 60 g and 30 g for men and women, respectively. In addition, the maximum average daily intake of ethanol can reach 150 g [58–60]. Approximately 7.103 g/kg alcohol was administrated in the present study, which is equivalent to a human dosage of 0.7805 g/kg [15]. Based on the average body weight of 70 kg, the total amount of alcohol scales to 54.638 g in the present study, which is close to the amount of alcohol reported earlier. BBR is able to prevent the damage inflicted at high doses, demonstrating that it could be used potentially as an effective therapy in clinical practice.

Conclusions

In summary, this is the first report comprehensively demonstrating that pretreatment with BBR before acute alcohol consumption protects the gastrointestinal mucosa from alcohol injuries. The oral administration of BBR could effectively prevent gastrointestinal damage. The mechanism by which BBR conferred protective effects included the regulation of inflammatory cytokine profiles by directly targeting gene transcription, including the genes encoding TLR2 and TLR4. This work provides a reasonable therapeutic strategy to protect against gastrointestinal damage induced by acute heavy alcohol consumption.

Supporting Information

S1 File. Figure A. mRNA expressions of inflammatory cytokines of mouse small intestines after oral administration of alcohol. Figure B. mRNA expressions of inflammatory cytokines of mouse stomach after oral administration of alcohol.

S1 Table. Primer sequence for q-PCR (mice).

S2 Table. Primer sequence for q-PCR (Caco2 cells).

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Author Contributions
Conceived and designed the experiments: XW FL LD. Performed the experiments: XW FL YC JJ XY. Analyzed the data: FD XW LD. Contributed reagents/materials/analysis tools: XJY FD. Wrote the paper: XW YW DX LD.

References
1. Patil JB, Kim J, Jayaprakasha GK (2010) Berberine induces apoptosis in breast cancer cells (MCF-7) through mitochondrial-dependent pathway. Eur J Pharmacol 645(1–3):70–78. doi: 10.1016/j.ejphar.2010.07.037 PMID: 20691179
2. Chidambara Murthy KN, Jayaprakasha GK, Patil BS (2012) The natural alkaloid berberine targets multiple pathways to induce cell death in cultured human colon cancer cells. Eur J Pharmacol 688(1–3):14–21. doi: 10.1016/j.ejphar.2012.05.004 PMID: 22617025
3. Hu J, Choi Y, Wang Y, Kheir MM, Li H, Yuan Z, et al. (2012) PI3K p55γ promoter activity enhancement is involved in the anti-apoptotic effect of berberine against cerebral ischemia–reperfusion. Eur J Pharmacol 674(2):132–142.
4. Zhang X, Wang C, Li Y, Dong L, Cui W, Wang L, et al. (2012) Neuroprotection of early and short-time applying berberine in the acute phase of cerebral ischemia: Up-regulated pAkt, pGSK and pCREB, down-regulated NF-kappa B expression, ameliorated BBB permeability. Brain Res 1459:61–70. doi: 10.1016/j.brainres.2012.03.065 PMID: 22560097
5. Hong JS, Chu YK, Lee H, Ahn BH, Park JH, Kim MJ, et al. (2012) Effects of berberine on hippocampal neuronal damage and matrix metalloproteinase-9 activity following transient global cerebral ischemia. J Neurosci Res 90(2):489–497. doi: 10.1002/jnr.22756 PMID: 22052603
6. Liu LZ, Cheung SC, Lan LL, Ho SK, Xu HX, Chan JC et al. (2010) Berberine modulates insulin signaling transduction in insulin-resistant cells. Mol Cell Endocrinol 317(1–2):148–153. doi: 10.1016/j.mce.2009.12.027 PMID: 20036710
7. Kong WJ, Wei J, Abidi P, Lin M, Inaba S, Li C, et al. (2004) Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med 10(12):1344–1351. PMID: 15531889
8. Lee CH, Chen JC, Hsiang CY, Wu SL, Wu HC, Ho TY (2007) Berberine suppresses inflammatory agents-induced interleukin-1β and tumor necrosis factor-alpha productions via the inhibition of IκB degradation in human lung cells. Pharmaco Res 56(3):193–201. PMID: 17681786
9. Domitrovic R, Jakovac H, Blagojevic G (2011) Hepatoprotective activity of berberine is mediated by inhibition of TNF-α, COX-2, and iNOS expression in CCl4-intoxicated mice. Toxicology 280(1):33–43.
10. Bhutada P, Mundhada Y, Bansod K, Tawari S, Patil S, Dixit P, et al. (2011) Protection of cholinergic and antioxidant system contributes to the effect of berberine ameliorating memory dysfunction in rat model of streptozotocin-induced diabetes. Behav Brain Res 220(1):30–41. doi: 10.1016/j.bbr.2011.01.022 PMID: 21262264
11. Wang YG, Kheir MM, Chai YS, Hu J, Xing DM, Lei F, et al. (2011) Comprehensive study in the inhibitory effect of berberine on gene transcription, including TATA box. PLoS One 6(8): e23495. doi: 10.1371/journal.pone.0023495 PMID: 21887260
12. Amasheh M, Fromm A, Krug SM, Amasheh S, Andres S, Zeitz M, et al. (2010) TNF-α-induced and berberine-antagonized tight junction barrier impairment via tyrosine kinase, Akt and NFκB signaling. J Cell Sci 123(23):4145–4155.
13. Lee IA, Hyun YJ, Kim DH (2010) Berberine ameliorates TNBS-induced colitis by inhibiting lipid peroxidation, enterobacterial growth and NF-κB activation. Eur J Pharmacol 648(1–3):162–170. doi: 10.1016/j.ejphar.2010.08.046 PMID: 20828350
14. Yan F, Wang L, Shi Y, Yan F, Wang L, Washington MK, et al. (2012) Berberine promotes recovery of colitis and inhibits inflammatory responses in colonic macrophages and epithelial cells in DSS-treated mice. Am J Physiol Gastrointest Liver Physiol 302(5):G504–514. doi: 10.1152/ajpgi.00312.2011 PMID: 22173918
15. Kheir MM, Wang YG, Hua L, Hu J, Li LL, Lei F, et al. (2010) Acute toxicity of berberine and its correlation with the blood concentration in mice. Food Chem Toxicol 48(4):1105–1110. doi: 10.1016/j.fct.2010.01.033 PMID: 20138204
16. Pan GY, Wang GJ, Liu XD, Fawcett JP, Xie YY (2002) The involvement of P-glycoprotein in berberine absorption. Pharmacol Toxicol 91(4):193–197. PMID: 12530470
17. Liu YT, Hao HP, Xie HG, Lai L, Wang Q, Liu CX, et al. (2010) Extensive intestinal first-pass elimination and predominant hepatic distribution of berberine explain its low plasma levels in rats. Drug Metab Dispos 38(10):1779–1784. doi: 10.1124/dmd.110.033936 PMID: 20694337
18. Cicero AF, Ertek S (2009) Metabolic and cardiovascular effects of berberine: from preclinical evidences to clinical trial results. Clin Lipidol 4(5):553–563.

19. Zuo F, Nakamura N, Akao T, Hattori M (2006) Pharmacokinetics of berberine and its main metabolites in conventional and pseudo germ-free rats determined by liquid chromatography/ion trap mass spectrometry. Drug Metab Dispos 34(12):2064–2072. PMID: 16956957

20. Coltart C, Anderson I, Barh B, Dewhurst N, Donohoe J, Dukat A, Gilmore I, et al. (2011) An international consensus for medical leadership on alcohol. Lancet 378(9798):1215.

21. Heberlein A, Kaser M, Lichteninghagen R, Rhein M, Lenz B, Kornhuber J, et al. (2014) TNF-α and IL-6 serum levels: Neurobiological markers of alcohol consumption in alcohol-dependent patients? Alcohol 48(7):671–676. doi: 10.1016/j.alcohol.2014.08.003 PMID: 25262503

22. Yazir Y, Tugay M, Utkan Z, Utkan T (2012) Effects of chronic ethanol consumption on rat upper gastrointestinal system: Functional and histologic findings. Alcohol 46(7):649–655. doi: 10.1016/j.alcohol.2012.06.003 PMID: 22818204

23. Liu MY, Chiang JPJ, Hsu DZ, Deng JF (2003) Abamectin attenuates gastric mucosal damage induced by ethanol through activation of vagus nerve in rats. Alcohol 30(1):61–65. PMID: 12878275

24. Shackleford C, Long G, Wolf J, Okerberg C, Herbert R (2002) Qualitative and quantitative analysis of nonneoplastic lesions in toxicity studies. Toxicol Pathol 30(1):93–96. PMID: 11890482

25. Chai YS, Hu J, Lei F, Wang YG, Yuan ZY, Lu X, et al. (2013) Effect of berberine on cell cycle arrest and cell survival during cerebral ischemia and reperfusion and correlations with p53/cyclin D1 and PI3K/Akt. Eur J Pharmacol 708:44–55. doi: 10.1016/j.ejphar.2013.02.041 PMID: 23499694

26. Jiang JF, Wang YG, Hu J, Lei F, Kheir MM, Wang XP, et al. (2013) Novel effect of berberine on thermoregulation in mice model induced by hot and cold environmental stimulation. PLoS ONE 8(1):e54234. doi: 10.1371/journal.pone.0054234 PMID: 23335996

27. Ueyama T, Saika M, Senba E (2001) Distinct gene expression in the stomach following stress and alcohol exposure. Kaibogaku Zasshi, 76(5):435–441. PMID: 11729671

28. Halleux C, Schneider YJ (1991) Iron-absorption by intestinal epithelial-cells: 1.Caco2 cells cultivated in serum-free medium, on polyethyleneterephthalate microporous membranes, as an in vitro model. In Vitro Cell Dev B 27(4):293–302.

29. Soellner R, Göbel K, Scheithauer H, Bräker AB (2014) Alcohol use of adolescents from 25 European countries. J Pub Health 22(1):57–65.

30. Room R, Babor T, Rehm J (2005) Alcohol and public health. Lancet 365(9458):519–530. PMID: 15705462

31. Bergmann MM, Rehm J, Klipstein-Grobusch K, Boeing H, Schutze M, Drogan D, et al. (2013) The association of pattern of lifetime alcohol use and cause of death in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Int J Epidemiol 42(6):1772–1790. doi. 10.1093/ije/dyt154 PMID: 24415611

32. Lehnert M, Kovacs EJ, Molina PE, Relja B (2014) Modulation of inflammation by alcohol exposure. Mediators of Inflammation 2014: e283756.

33. Morris NL, Ippolito JA, Curtis BJ, Chen MM, Friedman SL, Hines IN, et al. (2015) Alcohol and inflammatory responses: Summary of the 2013 Alcohol and Immunology Research Interest Group (AIRIG) meeting alcohol. Alcohol 49(1):1–6. doi: 10.1016/j.alcohol.2014.07.018 PMID: 25488277

34. Pang MY, Bala S, Kodys K, Catalano D, Szabo G (2011) Inhibition of TLR8- and TLR4-induced Type I IFN induction by alcohol is different from its effects on inflammatory cytokine production in monocytes. BMC Immunol 12:55 (doi:10.1186/1471-2172-12-55) PMID:21962237

35. Lippai D, Bala S, Csak T, Kurt-Jones EA, Szabo G (2013) Interleukin-1 alpha and IL-6 through a NO/cGMP dependent pathway. Alcohol Clin Exp Res 34(1):51–56. doi: 10.1111/j.1530-0277.2009.01065.x PMID: 19860807
40. Morrow CMK, Mruk D, Cheng CY, Hess RA (2010) Claudin and occludin expression and function in the seminiferous epithelium. Philos Trans R Soc Long B Biol Sci 365(1546):1679–1696.

41. Suzuki T (2013) Regulation of intestinal epithelial permeability by tight junctions. Cell Mol Life Sci 70 (4):631–659. doi: 10.1007/s00018-012-1070-x PMID: 22782113

42. Saitou M, Fujimoto K, Doi Y, Itcho M, Fujimoto T, Furuse M, et al. (1998) Occludin-deficient embryonic stem cells can differentiate into polarized epithelial cells bearing tight junctions. J Cell Biol 141:397–408. PMID: 9547818

43. Saitou M, Furuse M, Sasaki H, Schulzke JD, Fromm M, et al. (1998) Occludin-deficient embryonic stem cells can differentiate into polarized epithelial cells bearing tight junctions. Mol Biol Cell 11:4131–4142. PMID: 11102513

44. Furuse M, Fujita K, Hiiragi T, Fujimoto T, Tsukita S (1998) Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol 141:397–408. PMID: 9548718

45. Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S (1998) Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol 141:1539–1550. PMID: 9647647

46. Findley MK, Koval M (2009) Regulation and roles for claudin-family tight junction proteins. IUBMB Life 61(4):431–437. doi: 10.1002/iub.175 PMID: 19319969

47. Hou J, Gomes AS, Paul DL, Goodenough DA (2006) Study of claudin function by RNA interference. J Biol Chem 281:36117–36123. PMID: 17018523

48. Markov AG, Veshniakova AI, Krug S, Milatz S (2007) The tight junction proteins expression in the epithelium of the small intestines in a rat. Ross Fiziol Zh Im I M Sechenova 93(9):1043–1054. PMID: 18030802

49. Nagatake T, Fujita H, Minato N, Hamazaki Y (2014) Enteroendocrine cells are specifically marked by cell surface expression of claudin-4 in mouse small intestine. PLoS ONE 9(3):e90638.

50. Wang Y, Tong J, Chang B, Wang B, Zhang D (2014) Effects of alcohol on intestinal epithelial permeability and expression of tight junction-associated proteins. Mol Med Rep 9(6):2352–2356. doi: 10.3892/mmr.2014.2126 PMID: 24718485

51. Yuki T, Yoshida H, Akazawa Y, Komiya A, Sugiyama Y, Inoue S (2011) Activation of TLR2 enhances tight junction barrier in epidermal keratinocytes. J Immunol 187(6): 3230–3237. doi:10.4049/jimmunol.1100058 PMID: 21841130

52. Nagyoszi P, Wilhelm I, Farkas AE, Fazakas C, Dung NT, Hasko J, et al. (2010) Expression and regulation of toll-like receptors in cerebral endothelial cells. Neurochem Inter, 57(5): 556–564.

53. Ferrari L, Berard F, Debrauwer L, Chabo C, Langella P, Bueno L, et al. (2006) Impairment of the intestinal barrier by ethanol involves enteric microflora and mast cell activation in rodents. Am J Pathol 168:1148–1154. PMID: 16565490

54. Tamai H, Kato S, Horie Y, Ohki E, Yokoyama H, Ishii H (2000) Effect of acute ethanol administration on the intestinal absorption of endotoxin in rats. Alcohol Clin Exp Res 24: 390–394. PMID: 10776853

55. Suenaert P, Bulteel V, Lemmens L, Noman M, Geypens B, Van Assche G, et al. (2002) Antitumor necrosis factor treatment restores the gut barrier in Crohn’s disease. Am J Gastroenterol 97: 2000–2004. PMID: 12190167

56. Anderson P, Wojnar M, Jakubczyk A, Gual A, Reynolds J, Segura L, et al. (2014) Managing alcohol problems in general practice in Europe: Results from the European ODHIN survey of general practitioners. Alcohol Alcohol, 49(5):531–539. doi: 10.1093/alcalc/agu043 PMID: 25031247