Protective effect of monoammonium glycyrrhizinate on experimental colitis in Wistar rats

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Glycyrrhizic acid ammonium salt.

Glycyrrhizic acid (GA) is a triterpene glycoside, it consists of one molecule of glycyrrhetinic acid and two molecules of glucuronic acid. GA possesses wide spectrum of biological and pharmaceutical effects. It is the most important ingredient of the Glycyrrhiza species, family Leguminosae.
• Glycyrrhiza glabra L., G. uralensis Fisch., and G. inflata Batal are the three major species producing GA. They are predominantly found in Greece, Italy, Spain, Turkey, Central and South East Asian countries. The main source of GA are roots and stolon of licorice plants (Gycyrrhiza glabra). Their extracts have no calorific value and sweetness of about 50 times that of sucrose.

• Licoric extracts like the natural source of GA are used in pharmaceuticals, cosmetics, food additives like natural sweetener, confectionery products and tobacco flavors.
The pharmacological effects of GA include:

• antiinflammatory
• antiallergic
• antitumor
• antimicrobial
• antiviral effects (HIV, viral hepatitis, common cold)
• antioxidant
• antidiabetic
• antiulcer
• hepatoprotective etc.
This study was designed to evaluate the antiinflammatory activity of monoammonium glycyrrhizinate (GA) on experimental 2,4-dinitrobenzenesulfonic acid hydrate (DNBS)-induced colitis in Wistar rats.
Material and Methods

The experiments were approved by the Bulgarian Food Safety Agency (Protocol № 36/18.06.2015). The experimental protocol was conducted in compliance with the national laws and policies, in conformity with the international guidelines (EEC Council Directive 86/609, IL 358, 1, December 12, 1987).

Colitis was induced in accordance with the method described previously by Barbara et al. (2000).
• During a short anesthesia with a combination of ketamine (MSD, Animal Health) at a dose of 90 mg/kg and xylazine (Bioveta) at a dose of 30 mg/kg; 2,4-dinitrobenzenesulfonic acid (DNBS) in 0.25 ml of 50% ethanol was administered intrarectally via a polyethylene catheter inserted 8 cm proximal to the anus. Control rats received 0.25 ml of 50% ethanol.

• Animals received GA (100 or 200 mg/kg, dissolved in double distilled water, using Tween 60) intraperitoneally for 6 days, starting 1 day before colitis induction. Glycyrrhizic acid monoammonium salt (purity of 98.1%) was purchased from Sigma Aldrich Ltd.

• On day 6 colonic tissues were excised and scored for macroscopic and histological damages.
The macroscopic criteria were based on the following:

- presence of adhesions between colon and other intra-abdominal organs,
- consistency of colonic fecal material,
- thickening of colonic wall,
- macroscopic mucosal damage:

<table>
<thead>
<tr>
<th>Score</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Localized hyperemia, no ulcers</td>
</tr>
<tr>
<td>2</td>
<td>Ulceration without hyperemia or bowel wall thickening</td>
</tr>
<tr>
<td>3</td>
<td>Ulceration with hyperemia at one site</td>
</tr>
<tr>
<td>4</td>
<td>Two or more sites of ulceration and hyperemia</td>
</tr>
<tr>
<td>5</td>
<td>Major sites of damage extending &gt;1 cm along the length of colon</td>
</tr>
<tr>
<td>6</td>
<td>Area of damage extending &gt;2 cm along the length of colon</td>
</tr>
</tbody>
</table>
• **Microscopic criteria** were assessed by light microscopy on hematoxylin- and eosin-stained sections obtained from whole-gut specimens, taken from a region of inflamed colon.

• **Histological criteria** included: degree of mucosal architecture changes, cellular infiltration, external muscle thickening, presence of crypt abscess, and goblet cell depletion.

• All parameters of macroscopic and histological damage were recorded and scored for each rat by two observers blinded to the treatment.
• Blood samples were tested in duplicate by enzyme-linked immunosorbent assay (ELISA) for IL-1alpha, IL-6, and IL-10 and TNF-alpha (R&H Systems). Immunological research carried out according to methods described in the company's protocols.

• **Statistical analysis.** Data are presented as mean values ± S.E.M. and were tested by one-way ANOVA, followed by the LSD as a post-hoc test. A level of P<0.05 was considered significant. All analyses were performed using Statgraphics Centurion, SPSS 14 statistical software.
Results

Six days after DBSA administration were observed changes in body weight, decreased food intake, diarrhea, some times with blood present in the stool.
A significant body weight loss was recorded after colitis induction. Six days after DBSA administration rats in DBSA group and GA1 (100 mg/kg) group had a mean decrease of 32.5±5.3 g and 34.2±8.7 g in their body weight respectively, whereas rats in control and GA2 (200 mg/kg) group displayed a weight gain (16.7±3.2 g and 12.0±2.8 g, respectively).
Six days after DBSA administration, the distal part of colon appeared thickened and ulcerated with evident areas of inflammation. The intestinal damages were widespread, however the area of maximal changes is generally localized to the distal 4-5 cm of the colon. Adhesions were often present; the bowel was shorten (contr. - 15.67±0.33 cm; DBSA - 12.67±0.17 cm*; GA1 - 14.88±0.19 cm; GA2 - 15.27±0.26 cm†) with a macroscopic damage.
Results

Colonic ulcerations and inflammation - macroscopic view

DBSA group  GA1 group  GA2 group
Macroscopic scoring of colonic ulceration and inflammation.

* - p<0.05 vs. GA2 group; † - p<0.05 vs. DBSA group.
(0 - normal; 1 - localized hyperemia; 2 - ulceration without hyperemia; 3 - ulceration with hyperemia at 1 site; 4 - 2 or more site of ulceration; 5 - major site of damage extending >1 cm; 6 - area of damage extending >2 cm).
Consistency of fecal material as a indirect marker of diarrhea. Graph represents the results from DBSA-treated rats, either alone or in the presence of GA1 (100 mg/kg) or GA2 (200 mg/kg). Each column represents the mean (n=9). † - p<0.05 vs. DBSA group.
(1 - formed; 2 - loose; 3 - liquid stool).
Interleukin-1 level in plasma from control of DBSA-treated rats, either alone or in the presence of GA1 (100 mg/kg) or GA2 (200 mg/kg). Each column represents the mean (n=9). * - p<0.05 vs. control group; † - p<0.05 vs. DBSA group.
Results

Interleukin-6 level in plasma from control of DBSA-treated rats, either alone or in the presence of GA1 (100 mg/kg) or GA2 (200 mg/kg). Each column represents the mean (n=9). * - p<0.05 vs. control group; † - p<0.05 vs. DBSA group.
Interleukin-10 level in plasma from control of DBSA-treated rats, either alone or in the presence of GA1 (100 mg/kg) or GA2 (200 mg/kg). Each column represents the mean (n=9). * - p<0.05 vs. control group; † - p<0.05 vs. DBSA group.
Results

Tumor necrosis factors-alpha level in plasma from control of DBSA-treated rats, either alone or in the presence of GA1 (100 mg/kg) or GA2 (200 mg/kg). Each column represents the mean (n=9). * - p<0.05 vs. control group; † - p<0.05 vs. DBSA group.
Results

**Microscopic damage and inflammation** were assessed by light microscopy on hematoxylin/eosin-stained slides obtain from the whole gut specimens.

Histological criteria included mucosal architecture loss; cellular infiltrate; muscle thickening; crypt abscess and goblet cell depletion.
Results

Histological examination of DBSA-treated rats showed:

large areas of mucosal necrosis, where the glandular architecture was completely destroyed;

submucosa was thickened with edema and marked infiltration with inflammatory cells associated with vasodilation;

alterations of muscular layer, which appeared also thickened.

Hematoxylin eosin-stained section of colon form DBSA-treated rat (x40)
Results

Histological examination showed:

- Normal mucosal structure
- Granulation tissue (proliferating capillaries and chronic inflammatory infiltrate)
- Healing of ulcerative defect.

Hematoxylin eosin-stained section of colon form DBSA- and GA200mg/kg-treated rat (x40)
Conclusion

The DBSA-induced colitis is a model of bowel inflammation characterized by body weight loss, decreased food intake, diarrhea, some times with blood present in the stool, ulceration and typical histological changes.
Conclusion

Our findings indicate that GA (monoammonium glycyrrhizinate) at a dose of 200 mg/kg inhibits significantly colonic inflammatory damages in a rat model of inflammatory bowel disease.
Reference:


European Food Safety Authority. Scientific Opinion on the safety and efficacy of glycyrrhizic acid ammoniated when used as a flavouring for all animal species. EFSA Journal. 2015;13(1):3971


Thank you!