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Study of the Gastroprotective Activity of Some Derivatives of Glycyrrhetic Acid in Various Etiologic Damage of the Gastric Mucosa

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Abstract Improving the effectiveness of pharmacotherapy for diseases of the digestive system, including gastric ulcer (gastropathy) and duodenal ulcer is one of the pressing problems of gastroenterology. a comparative study of the effect on the gastric mucosa of derivatives of glycyrrhetic acid (18-dehydroglycyrrhetic acid - 18-DHA, 3-amino derivative of glycyrrhetic acid - 3-APGA, ammonium salt of glycyrrhetic acid - ASGA) in gastric lesions of various etiologies. The gastroprotective activity of 18-DHA, 3-APGA and ASGK was studied in white rats with various models of gastric mucosal lesions (GLC), as well as their effect on the intensity of free radical processes, the content of conjugated dienes and malondialdehyde was studied. Experimental studies have shown that 18-DHA, 3-APGK and ASGK in rats with different models of gastropathy clearly reduced the degree of damage to the coolant, manifested in a decrease in the number of strip-shaped-like and punctate erosions with a decrease in the Pauls index. In its pharmacological activity, 18-DHA was superior to other compounds. The mechanism of gastroprotective action is the presence of antioxidant properties in the studied compounds. 18-DHA can be recommended as a remedy for the prevention of lesions of the gastric mucosa.

Keywords Gastropathy, Glycyrrhetic acid derivatives, Antioxidants, Gastroprotectors

1. Introduction

Amongst the somatic diseases, one of the leading places is occupied by diseases of the digestive system, among which the most common are gastric ulcer and duodenal ulcer, chronic gastritis and etc. [1]. At the same time, the treatment of acute and chronic inflammatory diseases is the main problem of the practical medicine and, regardless of the type and localization of the inflammatory process, the most accessible and widespread method of treatment is application of non-steroidal anti-inflammatory drugs (NSAIDs) [2].

However, the taking of NSAIDs is associated with the development of side effects, especially from the side of gastrointestinal tract [3]. It is known that on the basis of the pathogenesis of gastric and duodenal ulcer lays the hypersecretion of hydrochloric acid. Therefore, drugs reducing the secretion of hydrochloric acid are mostly used in the treatment of gastropathy [4].

Recently, it is believed that it is necessary to use gastroprotective agents for the prevention of gastropathy, which increase the resistance of the gastric mucosa against the influence of aggressive factors [1]. The use of misoprostol in this regard was not effective enough [5,6]. Therefore, preparations from the roots of licorice (Glycyrrhiza glabra L.) are proposed for prevention of the damage of gastric mucosa, which has a wide range of pharmacological effects, such as anti-inflammatory, anti-allergic, antiproliferative [7].

In order to increase the pharmacological activity of licorice preparations, the derivatives of glycyrrhetic acid have been synthesized, including 18-dihydroglycyrrhetic acid (18-DHA), 3-amino derivatives of glycyrrhetic acid (3-ADGA), ammonium salt of glycyrrhetic acid (ASGA) and etc. Although the anti-inflammatory activity of these compounds have been studied sufficiently [8], but their effect on the state of the gastric mucosa has been insufficiently studied [9].

The purpose of current scientific work was a comparative study of the effect of some derivatives of glycyrrhetic acid on the gastric mucosa in the various etiological damage of it in rats.

2. Material and Methods

2.1. Experiments

The studies were carried out on male white rats weighing 175-195 g. The animals were kept in accordance with

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the rules of the European Convention for the Protection of Vertebrate Animals used for Experiments or for Other Scientific Purposes [Strasbourg, 1986], in compliance with the principles and Good Laboratory Practice (GLP). The study was approved by the local ethics Committee.

The experiments were carried out in 3 series: In the first series of studies, NSAIDs- induced gastropathy was modeled by a single intragastric administration of indomethacin at a dose of 60 mg/kg [9,10]. In this series of experiments, the investigated medicinal compounds were administered at the same dose of 50 mg/kg intragastrically for 7 days before modeling indomethacin-induced damage of the gastric mucosa (GF). The last administration of preparations was carried out 1 hour before the onset of the action of the ulcer-forming factor.

4 hours after the administration of the ulcer-forming agent, the animals were killed under ether anesthesia by one-stage decapitation, the stomach was removed, cleaned, washed with cold saline solution. The total as well as the number of strip-shaped-like and point erosions in the gastric mucosa (SM) were separately counted, and the Pauls index was calculated.

The stress-induced damage of gastric mucosa was reproduced in rats fasting for 48 hours by fixing the animals on the supine position for 24 hours at the second series of experiments [1]. The same doses of investigated preparations - 50 mg/kg were administered before the tying of rats' paws. Rats of the control group were administered an equal volume of drinking water.

After ending the immobilization period, the animals were sacrificed and their stomachs were carefully examined.

In the third series of experiments, the damage of the gastric mucosa was reproduced by applying mechanical irritation to the duodenal region (Anichkov S.V., Zavodskaya I.S., 1965). The abdominal cavity of rats was opened under ether anesthesia with aseptic conditions along the white line of the abdomen. Then, mechanical irritation was applied to the duodenal area with the Pean's hemostatic clamp for 10 minutes. After mechanical irritation, the abdominal wound was sutured in layers. After 24 hours, the animals were sacrificed and the stomach wall was subjected to macroscopic examination. The investigated preparations were administered immediately before the mechanical irritation of duodenal region.

For the integral assessment of the level of free radicals of oxidation, we used the method of biochemiluminescence determination. The intensity of the flash was determined using a biochemiluminometer BKhL-06M. The determination of content of the primary products of LPO-diene conjugates was carried out on spectrophotometer at 233 nm wavelength (Inesa 752N, Shenzhen Mindray Bio Medical) [8]. The content of TBA-active LPO products was determined by the method of Andreev L.I. [10].

2.2. Statistical Analysis

The data obtained were processed by the method of variation statistics using the paired Student's test and one-way analysis of variance using the standard software package BIOSTAT 2009 with an assessment of the significance of indicators (M ± m). Differences in the compared groups were considered significant at a significance level of 95% $p < 0.05$, in the case of $0.05 < p < 0.10$, the differences were assessed as a trend.

3. Results and Discussion

According to the requirements for experimental studies for new compounds with the supposed antiulcerogenic action, it is required to conduct tests on various models of damage of the gastric mucosa. It is known that the most frequent and dangerous complication of non-steroidal anti-inflammatory drugs is the development of severe gastropathy [5,7,9].

Proceeding from this, we carried out studies to investigate the influence of compounds to the course of indomethacin-induced gastropathy in the first series of experiments. The results of the experimental studies showed that under the influence of indomethacin, there was damage of gastric mucosa in all animals of control group. At the same time, the number of strip-shaped and point erosion was practically the same, and the Pauls index was equal to 9.3. In contrast, in the group of rats treated with 18-dihydroglycyrrhetic acid, the number of lesions decreased by 65.5%, and the number of strip-shaped-like and point erosions decreased by 89 and 42.4%, respectively. Pauls index in this group of animals decreased by 82%. It is noteworthy that there was damage of gastric mucosa at 33% animals of this group, where the area of damage was less by 81.4%.

Therefore, 18-dihydroglycyrrhetic acid has a distinct gastroprotective effect in indomethacin-induced gastropathy model. It can be seen from the data in table 1 that 3-amino derivatives of glycyrrhetic acid and ammonium salt of glycyrrhetic acid in the model of NSAID-induced gastropathy have a similar effect. Under the influence of 3-amino derivatives of glycyrrhetic acid and ammonium salt of glycyrrhetic acid, the amount of damage of gastric mucosa decreased by 43.3% and 6.6%, compared with the control group, respectively. At the same time, the number of strip-shaped and point erosion was less by 49.1% and 7.4%, as well as 36.9% and 3.8%, respectively, although the number of animals with damage of gastric mucosa was only 33% in the group of animals treated with 3-amino derivatives of glycyrrhetic acid and 68% in group treated with the ammonium salt of glycyrrhetic acid. The Pauls index was 6.76 and 7.14 in these groups, respectively. It is noteworthy that, the area of damage decreased by 67.2% in rats treated with 3-amino derivatives of glycyrrhetic acid and by 32.3% in rats treated with the ammonium salt of glycyrrhetic acid. It can be seen that the antiulceragenic activity of 3-amino derivatives of glycyrrhetic acid and ammonium salt of glycyrrhetic acid was inferior to 18-dehydroglycyrrhetic acid.

Table 1. The influence of some derivatives of glycyrrhetic acid on the state of the gastric mucosa in gastropathy induced by indomethacin (M±m)

Groups	Number of lesion of gastric mucosa	Number of strip-shaped erosion	Number of point erosion	Pauls index	Rats with ulcers, %	Damage area in mm^2
Control	9,30±1,61	4,61±0,89	4,69±1,23	9,3	100	1,61±0,30
18-DHA	3,21±0,93*	0,51±0,09*	2,70±0,69	1,60	33	0,30±0,12*
3-ADGA	5,27±1,24	2,31±0,42*	2,96±0,73	6,76	33	0,52±0,15*
ASGA	8,78±0,87	4,27±0,56	4,51±0,37	7,14	68	1,09±0,33

Note: * - statistically significant differences compared to control.
18-DHA – 18-dehydroglycyrrhetic acid. 3-ADGA – 3-amino derivative of glycyrrhetic acid.
ASGA – ammonium salt of glycyrrhetic acid.

Table 2. The effect of some derivatives of glycyrrhetic acid on the state of the gastric mucosa in immobilization stress (M±m)

Groups	Number of lesion of gastric mucosa	Number of strip-shaped erosion	Number of point erosion	Pauls index	Rats with ulcers, %	Damage area in mm^2
Control	6,14±0,55	1,31±0,47	4,61±0,67	3,34	100	1,48±0,18
18-DHA	2,13±0,70*	0,83±0,12	1,30±0,32*	0,49	45	0,91±0,10
3-ADGA	2,78±0,62*	0,91±0,14	1,87±0,22*	0,84	56	1,20±0,14
ASGA	3,06±0,57*	1,11±0,15	1,95±0,29*	1,20	67	1,43±0,24

Note: * - statistically significant differences compared to control.
18-DHA – 18-dehydroglycyrrhetic acid. 3-ADGA – 3-amino derivative of glycyrrhetic acid.
ASGA – ammonium salt of glycyrrhetic acid.

Table 3. The effect of some derivatives of glycyrrhetic acid on the state of the gastric mucosa at trauma of the duodenal region (M±m)

Groups	Number of lesion of gastric mucosa	Number of strip-shaped erosion	Number of point erosion	Pauls index	Rats with ulcers, %	Damage area in mm^2
Control	18,3±1,03	6,6±0,95	11,7±1,04	7,30	100	42,5±4,1
18-DHA	6,91±0,48*	2,7±0,19*	4,21±0,63*	0,80	47	22,0±2,6*
3-ADGA	10,10±0,91*	3,9±0,62	6,2±1,4*	0,96	63	21,2±2,2*
ASGA	14,2±1,22	5,0±0,21	9,2±1,7	1,34	68	29,3±3,0

Note: * - statistically significant differences compared to control.
18-DHA – 18-dehydroglycyrrhetic acid. 3-ADGA – 3-amino derivative of glycyrrhetic acid.
ASGA – ammonium salt of glycyrrhetic acid.

Table 4. The effect of some derivatives of glycyrrhetic acid on the intensity of chemiluminescence, the content of conjugated dienes and malondialdehyde in indomethacin-induced damage to the gastric mucosa (M ± m)

Groups	Chemiluminescence intensity, impulse / sec	Conjugated dienes (D233) mg lipids	Malondialdehyde, nmol/mg protein
Intact	35,7±1,19	0,6±0,015	0,35±0,014
Control	75,7±2,14*	0,91±0,02*	1,18±0,033*
18-DHA	43,5±1,3*#	0,63±0,17#	0,42±0,043#
3-ADGA	46,9±1,88*#	0,7±0,018*#	0,45±0,013*#
ASGA	53,0±1,53*#	0,78±0,023*#	0,49±0,017*#

Note: * - statistically significant differences compared to control.
18-DHA – 18-dehydroglycyrrhetic acid. 3-ADGA – 3-amino derivative of glycyrrhetic acid.
ASGA – ammonium salt of glycyrrhetic acid.

Another important pathogenetic factor in development of gastropathy is stress. The thermal injuries, myocardial infarction, after surgical intervention and etc. cause to the development of stress, which may lead to damage of gastric mucosa [12,13]. A model of immobilization stress is also used in experimental studies for the investigation of antiulcer activity of medicines. The results of studies have shown that the immobilization of animals leads to severe damage of gastric mucosa. Damage of gastric mucosa was observed in all animals of the control group more or less, so a total damage of area was 1.48±0.73 mm2. In this case, the number of strip-shaped erosion was 1.31±0.47, and point erosion - 4.61±0.67. Consequently, the development of stress during the immobilization of animals leads to severe gastropathy. It is known that the mechanism of this condition is due to increased activity of the corticoadrenal system [13].

Unlike, in animals receiving the ammonium salt of glycyrrhetic acid, the number of rats with damage of gastric mucosa decreased by 2 times, and the number of

strip-shaped and point erosions also decreased by 15.3, and 57.7%, which caused to decrease of the area of damage by 3.4%, although the number of rats with ulcers was 67%, and the Pauls index decreased by 64.1%.

We noted a higher gastroprotective activity in the group of animals that received the 3-amino derivative of glycyrrhetic acid, and especially 18-dehydroglycyrrhetic acid. Thus, the amount of damage of gastric mucosa in animals of these groups decreased by 54.7% and 65.3%, and the number of point erosions was 59.4 and 71.8%, respectively. It is noteworthy that the number of strip-shaped erosions decreased by 30.5% and 36.6% in animal, which received the 3-amino derivative of glycyrrhetic acid and 18-dehydroglycyrrhetic acid, respectively. As it can be seen from the data in the table 2, the number of rats with ulcers decreased by 56% and 45% in these groups, respectively. Thus, the studied derivatives of glycyrrhetic acid have a distinct gastroprotective effect in immobilization stress model. According to the results of morphological studies by M.A. Mamajanova (2016), there was a more accelerated and complete epithelialization of the wound surface under the influence of 18-dehydroglycyrrhetic acid, the epithelium and connective tissue differentiated earlier, and the muscle layers of the injured stomach wall were restored, which indicates the stimulating and regulating effect of the medicines to the main stages of the regeneration process (restructuring, proliferation, differentiation). 18-dehydroglycyrrhetic acid might be used for the prevention and treatment of peptic ulcer disease, as well as after stomach surgery.

In the next series of experiments, we studied the antiulcer activity of the medicines by the method (I.S. Zavodskoy, 1954, 1955), in which was showed the possibility of obtaining experimental dystrophy of the stomach wall by applying various stimuli - electrical, chemical and mechanical on the duodenal region. Wherein, destructive changes in the stomach wall in form of hemorrhages, erosions and ulcers occur 6-12 hours after irritation. Consequently, this model of experimental gastric ulcer is considered as an ulcer of neurogenic origin, and disturbances of the nervous regulation of trophic processes in the stomach wall play a decisive role in their genesis.

Using this model, we decided to clarify the question of the possible effect of glycyrrhetic acid derivatives on neurogenic dystrophy of the stomach wall.

The results of carried out studies on the gastroprotective effect of glycyrrhetic acid derivatives in trauma of the duodenal region showed that the investigated compounds had a distinct property for protection of the gastric mucosa from damage. Thus, the number of rats with ulcers decreased till 47%, 63% and 68%, respectively, in the groups receiving 18-dehydroglycyrrhetic acid, 3-amino derivative of glycyrrhetic acid and ammonium salt of glycyrrhetic acid. At the same time, the area of damage decreased by 82.4, 83.0 and 76.6%, respectively, in the indicated groups. As can be seen from the data in Table 3, the therapeutic effectiveness of the studied compounds manifested not only in a decrease of the damage of gastric mucosa, but also in a decrease of the number of strip-shaped and point erosions, which led an almost nine-fold decrease of the Pauls index.

Summarizing the obtained results, we can conclude that glycyrrhetic acid derivatives exhibit a distinct gastroprotective effect not only in the case of damage of the gastric mucosa induced by NSAIDs and stress, but also in trauma of the gastroduodenal region of the gastrointestinal tract. It can be seen that in its pharmacological activity 18-dehydroglycyrrhetic acid is superior to the rest of the studied compounds.

The mechanism of the gastroprotective action of the studied compounds is likely to be the suppression of the free radical process, which is one of the main links in the pathogenesis of the development of gastropathy in the studied extreme conditions [13,14,15].

An increase the activity of lipid peroxidation (LPO) and the content of their metabolic products in many diseases, including ulcerative process, served as the basis for studies to evaluate the influence of glycyrrhetic acid derivatives to LPO parameters in the indomethacin-induced damage of gastric mucosa [4,7]. For this purpose, the intensity of chemiluminescence and the content of lipid hydroperoxides (conjugated dienes (CD) of malondialdehyde (MDA)) were determined. The analysis of the results of the conducted biochemical studies showed that there was a more than twofold (by 112%) increase of the intensity of chemiluminescence in indomethacin-induced damage of gastric mucosa, which indicates an increase of the formation of free radicals. It is known that free radicals enhance lipid peroxidation of biological membranes of cells, as a result of which the concentration of oxidative stress products increases. Indeed, in control animals, the concentration of conjugated dienes in the blood increased in comparison with intact animals (by 52%, and malondialdehyde by 237.1%). Consequently, indomethacin-induced gastropathy was accompanied by a significant increase of the intensity of free radical lipid oxidation process. From a pathogenetic point of view, this fact is one of the important links of the pathogenesis of damage of gastric mucosa. Taking into account that the studied derivatives of glycyrrhetic acid have a distinct gastroprotective effect and it can be assumed about their properties to suppress the degree of oxidative stress.

It can be seen From the data in table 4 that the intensity of chemiluminescence decreased by 43%, 38% and 30% under the influence of 18-dehydroglycyrrhetic acid, a 3-amino derivative of glycyrrhetic acid and ammonium soliglycyrrhetic acid, respectively. It is noteworthy that derivatives of glycyrrhetic acid statistically significantly suppressed the rate of formation of free radicals. Under the influence of these compounds, the concentration of conjugated dienes decreased by 30.8%, 23.1% and 14.3% as well as malondialdehyde reduced by 64.4%, 61.9% and 58.5% Summarizing the above, we can conclude that the studied derivatives of glycyrrhetic acid have a high

antioxidant activity, among them,18-dehydroglycyrrhetic acid had more expressed efffect.

Thus, the mechanism of the gastroprotective action of glycyrrhetic acid derivatives is due to the suppression of the intensity of free radical processes, i.e. with their antioxidant activity.

4. Conclusions

1. The gastroprotective effect of glycyrrhetic acid derivatives was established in rats with various models of gastropathy.
2. 18-dehydroglycyrrhetic acid is more active than the 3-amino derivative of glycyrrhetic acid and the ammonium salt of glycyrrhetic acid particularly in indomethacin-induced gastropathy.
3. The mechanism of the gastroprotective action of the studied derivatives of glycyrrhetic acid is due to the suppression of the intensity of free radical processes.

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