Investigation of 4-methyl-2.2-dioxo-1H-2λ6,1-benzothiazine-3-carboxamide derivative influence on the biochemical markers of gastric mucosa in rats

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Non-steroidal anti-inflammatory drugs occupy a leading position in the pharmaceutical market, but their class-specific side effect (ulcerogenic action) significantly limits their widespread use. The N-(4-methoxybenzyl)-4-methyl-2,2-dioxo-1H-2λ6,1-benzothiazine-3-carboxamide derivative (compound NI-9) showed a pronounced analgesic effect in various models of pain syndromes. The aim of the study was to determine the effect of a new original derivative of 4-methyl-2,2-dioxo-1H-2λ6,1-benzothiazine-3-carboxylic acid on the macroscopic state and biochemical parameters of the gastric mucosa of rats. The study was performed on 51 male Wistar rats. Compound NI-9 and the reference drug meloxicam were administered intragastrically once daily for 28 days at doses of 3 and 5 mg/kg, corresponding to their ED50 analgesic activity. Macroscopic indicators of GM damage, glycosaminoglycan content, phospholipid profile, level of MDA and CGP, as well as nitrates and nitrites and H2S were determined. The results were processed in the program STATISTICA 10.0 using non-parametric methods. The results showed that the carboxamide derivative was safer for the stomach because the ulcer index of compound NI-9 was 1.73 times lower than that of meloxicam. The damaging effect of the compound was more pronounced at the pre-epithelial and epithelial levels of GM protection, while the post-epithelial level (production of vasodilating molecules NO and H2S) was practically unaffected by this derivative, unlike meloxicam, which caused damage at all levels of protection. The obtained data supplement the data on the pharmacodynamics of the 4-methyl-2,2-dioxo-1H-2λ6,1-benzothiazine-3-carboxylic acid derivative and determine the expediency of its further studies as a potential non-opioid analgesic.

Keywords: N-(4-methoxybenzyl)-4-methyl-2,2-dioxo-1H-2λ6,1-benzothiazine-3-carboxamide, ulcerogenic effect, gastric mucosa, biochemical markers, oxidative stress, nitrogen monoxide, hydrogen sulfide.

ARTICLE INFO
Received: 4 November 2020
Accepted: 4 December 2020

UDC: 615.015.35:615.276:547.728.2

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Introduction
Analysis of the pharmaceutical market of drugs shows that in the arsenal of modern non-narcotic drugs with pronounced analgesic and anti-inflammatory activity an important place belongs to the derivatives of carboxylic acids [9, 16]. According to the chemical structure, drugs of this class can be divided into several groups: first of all - derivatives of well-known salicylic acid (aspirin, etc.), derivatives of anthranillic and 2-aminonicotinic acids, which have structural similarity to salicylic acid (mefenamic, flufenamic acid, etc.). Particularly popular analgesics have been developed based on phenylacetic acid (diclofenac). In addition, the largest group of drugs approved for use as analgesics were 2-phenylpropionic acids (naproxen, dexketoprofen, ibuprofen). Separate mention should be made of derivatives of succinic acid (fenbufen and suxibuzone), heterocyclic acid (etodolac and ketorolac), and heterocyclic acid (indomethacin, sulindac). All of these drugs are the most common group of over-the-counter drugs used by more than 30 million people worldwide, despite the well-known class-specific gastrointestinal side effects (GI) associated with the free carboxyl group. However, there is no doubt that the presence of an acid fragment (or chemical group that can be easily converted to such in vivo), often has a very positive effect on the
biological properties of a molecule [18]. Therefore, carboxylic acids are always of particular interest for the search for promising analgesics [22], and their possible side effects can be eliminated by subsequent chemical transformations into labile non-acidic groups or special conditions of their introduction [12].

Given the above, our attention was drawn to new derivatives of 4-methyl-2,2-dixo-1H-2λ6.1-benzothiazine-3-carboxylic acid, in particular N-4-methoxybenzyl-amide - a substituted derivative (compound with laboratory code NI-9), which demonstrated high anti-inflammatory and analgesic activity in various models of pain perception [21]. Because this compound is derived from a biosynthetic substitution in the molecule of oxycamins, known nonsteroidal anti-inflammatory drugs (NSAIDs), in addition to establishing its pharmacological activity, it was necessary to conduct a study of the safety of this molecule in the gastrointestinal tract.

The aim of the study was to determine the effect of a new original derivative of 4-methyl-2,2-dixo-1H-2λ6.1-benzothiazine-3-carboxylic acid on the macroscopic state and biochemical parameters of the gastric mucosa of rats.

Materials and methods

The research was performed on the basis of a research laboratory for preclinical study of pharmacological substances of the Department of Pharmacology of National Pirogov Memorial Medical University, Vinnytsya (certificate of the Ministry of Health on technical competence №030/18 dated November 1, 2018, valid until October 31, 2023). The study included 51 male Wistar rats obtained from the vivarium of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. During the experiment, the animals were kept in standard conditions (day/night regime 12/12 and access to water and food ad libitum), according to the norms. The research met all the necessary requirements for humane treatment of experimental animals and complied with the rules of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and current laws of Ukraine. Compound NI-9 and the most chemically similar drug meloxicam were administered intragastrically once daily to animals against 85.7 % in the group administered the comparison drug. Its long-term (28-day) administration caused less pronounced damage to GM than the compound under study.

Results

Our results showed that long-term administration of the compound under study did not cause changes in eating behavior, appearance of rats and did not lead to death. Weight gain in animals administered NI-9 was 11.3±1.2 %, which was almost the same as in the meloxicam group (11.4±0.6 %). Body weight in control rats increased by 11.3±1.2 % during this period, but these differences were not significant. Analyzing the macroscopic picture of the gastric mucosa, it should be noted that the compound NI-9 caused less pronounced damage to GM than the comparison drug. Its long-term (28-day) administration caused ulcerative lesions was observed in only 71.4 % of animals against 85.7 % in the group administered meloxicam. The multiplicity of ulcers on the background of the introduction of a derivative of 4-methyl-2,2-dixo-1H-2λ6.1-benzothiazine-3-carboxylic acid was 54.2 %, and the...
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Specific gastric lesions, which include non-ulcer dyspepsia, epigastric pain, anorexia, esophagitis, constipation and diarrhea, erosions and ulcers in the stomach and duodenum, as well as perforation, have become clinically defined and are now referred to as "NSAID-gastropathy" [1, 2, 15, 17, 27]. These lesions mainly affect the stomach and duodenum, although NSAIDs can affect any part of the gastrointestinal tract, from the esophagus to the colon. The largest lesions are ketorolac, aceclofenac, celecoxib, desketoprofen, meloxicam, nimesulide and rofecoxib. The risk of bleeding was increased in patients with a history of peptic ulcer disease and/or gastrointestinal bleeding and using antiplatelet agents.

Nonsteroidal anti-inflammatory drugs are usually able to disrupt the integrity of the gastric mucosa due to the impact on different levels of its protection [6]. The main pre-epithelial (mucosal bicarbonate-phospholipid "barrier", creating cells of the superficial epithelium of the stomach, which produce mucus, bicarbonates, phospholipids, peptides, heat shock proteins, and some other biologically active substances that neutralize and disperse gastric lumen to mucus cells); epithelial (resistance of the surface of epithelial cells and intercellular contacts to the back diffusion of hydrogen ions and hydrophobic properties of the mucous membrane, which contribute to the "repulsion" of hydrochloric acid, as well as the high proliferative capacity of epithelial cells) and postepithelial, which are provided by vasoactive molecules. The most well-known components in this regard are prostaglandins (PG) - PGE2 and PGI2. According to modern ideas, their protective effect on the central nervous system is realized through several mechanisms: inhibition of hydrochloric acid in the stomach, increased secretion of mucus and bicarbonate, and PGI2 (prostacyclin), is a powerful vasodilator that provides normal blood circulation in the gastric mucosa, stabilizes the membranes of mast cells and lysosomes, inhibits the production of free radicals and enzymes by neutrophils, regulation of vascular endothelium.

Microcirculation disorders due to hyperproduction of vasoconstrictor and decrease in the content of vasodilating molecules play an important role in the biochemical mechanisms of development of NSAID-induced gastric lesions. The literature describes a number of biologically active substances of endothelial and plasma origin, which regulate vascular tone. Among these mediators, in addition to prostaglandins, oxygen free radicals, nitric oxide, and H$_2$S have recently been identified [4, 13, 24, 25, 28]. Therefore, the search for and research of new drugs that have anti-inflammatory and analgesic activity, it is necessary to establish their safety in the gastrointestinal tract.

The results obtained in our study showed that among the molecular mechanisms of modulation of the GM on the background of the compound NI-9, we can note its negative impact on the processes of lipid and protein peroxidation, mucus production, phospholipid spectrum of GM cell membranes, the severity of which meloxicam. In addition, it should be noted that in contrast to the latter, the derivative of benzothiazine-3-carboxylic acid has no inhibitory effect on the production of nitrogen monoxide, and also tends to increase the content of hydrogen sulfide in the GM, which may have been one of the explanations for its greater stomach safety.

The reason for such differences, in our opinion, may be the peculiarities of the chemical structure of this molecule. It is known from the literature that one of the prerequisites for the safety of NSAIDs in the gastrointestinal tract is a selective effect on PTGS2 and the absence or minimal effect on PTGS1. Meloxicam, as the closest in structure to NSAIDs, belongs to a group of drugs that mainly affect the inducible isoform of cyclooxygenase [3, 7]. The drug binds to the upper part of the PTGS2 channel and has a balanced PTGS2 selectivity profile. Other highly selective PTGS2-coxib inhibitors also do not have the ability to damage the stomach, however, they bind to the lateral pocket of the PTGS2 channel and inhibit thromboxane less, which explains the increased risk of thromboembolic complications if long-term use [5]. It is possible that the N-(4-methoxybenzyl)-4-methyl-2,2-dixo-1H-2λ$^6$.1-benzothiazine-3-carboxylic acid derivative studied has its own interactions with molecular markers of inflammation and pain. However, for final conclusions it is necessary to conduct additional research in this direction.

**Conclusion**

The data obtained show, that compound N-(4-methoxybenzyl)-4-methyl-2,2-dixo-1H-2λ$^6$.1-benzothiazine-3-carboxylic acid has a high degree of safety in relation to the gastrointestinal tract in intact, which exceeds reference preparation meloxicam. This difference is mainly due to a less pronounced depressant effect on the postepithelial mechanism of protection of the gastric mucosa, namely, the production of vasodilating molecules in the central nervous system. The results supplement the data on the pharmacodynamics of the 4-methyl-2,2-dixo-1H-2λ$^6$.1-benzothiazine-3-carboxylic acid derivative and determine the feasibility of its further studies as a potential non-opioid analgesic.

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