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EXPERIMENTAL STUDY OF STATE OF GASTRIC MUCOSA IN RATS UNDER INTRODUCTION OF NEW DERIVATE OF 4-[4-OXO-4H-QUINAZOLINE-3-YL] BENZOIC ACID (PC-66)

© H. Kramar

Скринінгові дослідження показали, що нове похідне 4- [4-оксо-4Н-хіназолін-3-іл] бензойної кислоти (сполука ПК-66) має значні антиноцицептивну та слабо виражену протизапальну і жарознижувальну активність на різних моделях болю та запалення. Стан захисного бар'єра слизової оболонки шлунка під впливом ПК-66 раніше не вивчався.

***Мета** даного дослідження полягала в тому, щоб вивчити здатність ПК-66 в порівнянні з диклофенаком натрію проявляти пошкоджуюче дію на слизову оболонку шлунка як у інтактних тварин, так і у щурів з ад'ювантним артритом, а також визначити роль впливу на простагландин-Н-синтазу і стабільних метаболітів оксиду азоту в патогенезі цих пошкоджень.*

***Матеріали та методи:** Вплив сполуки ПК-66 і диклофенаку натрію був досліджений на макроскопічних змінах слизової оболонки шлунка в умовах тривалого застосування та експериментального запалення.*

Спектрофотометричним методом, в гомогенатах СОШ щурів, за накопиченням окисленої форми донору електронів адреналіну, визначали активність простагландин-ендопероксид синтази (PGH-синтази). Сумарний вміст нітритів та нітратів, визначали за реакцією з реактивом Грісса, після попереднього відновлення нітратів суспензією цинкового порошку в розчині аміаку.

***Результати і обговорення.** Нами встановлено, що сполука ПК-66, на відміну від референс-препарату, не викликала суттєвих уражень шлунка за умов тривалого введення інтактним щурам.*

На тлі дії диклофенаку, активність PGH - синтетази в СОШ вірогідно знизилась відносно контролю, в той час як під впливом ПК-66, активність даного ферменту практично залишалась не змінною.

Вплив сполуки ПК-66 на щурів, на відміну від диклофенаку, був пов'язаний зі значним збільшенням стабільних метаболітів NO в СОШ, в той час як цей показник знизився в порівнянні з контрольною групою під впливом досліджуваного НПЗЗ.

***Висновки:** Нове похідне 4- [4-оксо-4Н-хіназолін-3-іл] бензойної кислоти не проявляє пошкоджуючої дії на слизову оболонку шлунка як у інтактних тварин, так і у щурів з експериментальним запальним процесом. В основі безпечності його щодо СОШ лежить відсутність у сполуки ПК-66, на відміну від диклофенаку натрію, інгібуючого впливу на продукцію вазодилатуючих молекул в СОШ*

***Ключові слова:** похідне 4- [4-оксо-4Н-хіназолін-3-іл] бензойної кислоти, гастротоксичність, PGH – синтетази, метаболіти NO*

1. Introduction

Pain is possibly the most unpleasant sensation our senses can detect.

Non-opioid analgesics and nonsteroidal anti-inflammatory drugs have one of the first places in the treatment of inflammatory and pain syndromes as showing a strong anti-inflammatory and analgesic effects [1]. The figures show that the millions of people in the world take drugs of this group annually, with a significant number of which have long-term use. Prolonged use of drugs these groups causes a number of side effects – gastro-, hepato- and nephrotoxicity, allergic reactions, disorders of the cardiovascular system, blood, etc .

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

It has been recognized that optimizing pain management in people with different inflammatory, somatic or other pathological conditions requires an individualized approach which seeks to maximize pain relief but minimize the risk of adverse drug reactions. [2].

One of the most important adverse reaction on non-opioid analgetics and nonsteroidal anti-anflammatory drugs is gastrotoxicity [3].

3. Analysis of recent studies and publications in which a solution of the problem and which draws on the author

According to the research literature, 4-oxo (amino-) quinazoline derivatives have shown cerebro- and actoprotective and antinociceptive properties, and appeared to be low-toxic substances [4–6].

It was shown that the new derivate of 4-[4-oxo-4H-quinazoline-3-yl] benzoic acid (PC-66) possess significant antinociceptive and weakly expressed anti-inflammatory and antipyretic activity on different models of pain and inflammation [7].

4. Allocation of unsolved parts of the general problem, which is dedicated to the article

The condition of gastric defense under influence of PC-66 is unknown.

This became the basis for further in-depth study effect compound PC-66 on molecular mechanisms that

protect the mucosa and influence healing at the cellular level [8].

5. Formulation of goals (tasks) of Article

The aim of this study was to explore the ability of PC-66 in comparison with diclophenac sodium produce gastric lesions on intact and rats with adjuvant arthritis, and to determine the role of influence on prostaglandin–H-synthase and stable metabolits of nitric oxide in the pathogenesis of these damages.

6. Statement of the basic material of the study (methods and objects) with the justification of the results

Experiments were conducted on male, Wistar rats weighing 180–220 g obtained from vivarium of Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. The rats were fed standard chow and water ad libitum, and were housed in a room with controlled temperature (22±1 °C), humidity (65–70 %) and light cycle (12 h light/12 h dark). The animal experimentation described in this study was conducted in accordance with internationally accepted principles for laboratory animal use and care. The study protocol was approved by the Ethical Committee of Vinnitsa national Pirogov Memorial medical university. Polyarthritis was induced in rats via an injection into right hind limb of 0,1 ml of Freund's Complete Adjuvant (Sigma, USA).

The rats were randomly assigned to several groups (n =7 per group) according to the treatment received. Intact rats were treated ones a day with: group I: 0.9 % NaCl saline (Control) was applied by intraperitoneal route (i.p.); group II: 8 mg/kg i.p. diclophenac ("Dicloberl"), Berlin-Chemie AG; group III: 1 mg/kg PC-66 by intraperitoneal route (i.p.). Rats with adjuvant arthritis received: group I: 0.9 % NaCl saline (Control, i.p.); group II: AA without treatment (positive control); group III: 4 mg/kg i.p. diclophenac; group IV: 1 mg/kg PC-66 (i.p.). The dosage schemes and treatment route were selected in accordance with previous successful experiments (ED50 according to the screening of analgesic activity). On the 28-30-th day of experiments for further investigations rats were euthanized by cervical dislocation. The stomachs were removed, cut open along the small curvature and flushed gently with lukewarm 0,9 % NaCl solution. Thereafter, the gastric mucosa was observed for lesions with the aid of a magnifying glass and the severity of the mucosal lesions scored. We estimated: inflation, hemorragias, hyperemia, smoothness of gastric mucosa, number of ulcers per animal, the degree of ulceration in points (1–5) was determined according to the following scale:

0 – no visible damage;

1 – the presence of edema or hemorrhage,

1-3 – small ulcers;

2 – several (more than 3) small ulcers or one large ulcer size;

3 – ulcer considerable size (diameter 4 mm);

4 – several large ulcers;

5 – breakthrough ulcer.

The mean ulcer index (MUI) for each group calculated as follows [9]:

$$MUI=\frac{\text{Total degree of ulceration}*\%\text{ of ulcerated rats}}{100\,\%}$$

The impact of PC-66 compound on the activity of PGH – synthetase and level of stable NO metabolites in gastric mucosa compared to diclofenac was assessed in experiments on 32 white mature male rats weighing 180–215 g, which were randomly divided into 4 groups (n=8):

Group I – control – (the animals received physiologic saline NaCl during a 3-day fasting);

Group II – the animals received PC-66 at a dose of 1 mg/kg/peritoneally during a 3-day fasting;

Group III – the animals received diclofenac sodium at a dose of 8 mg/kg intraperitoneally during a 3-day fasting;

Group IV – intact animals.

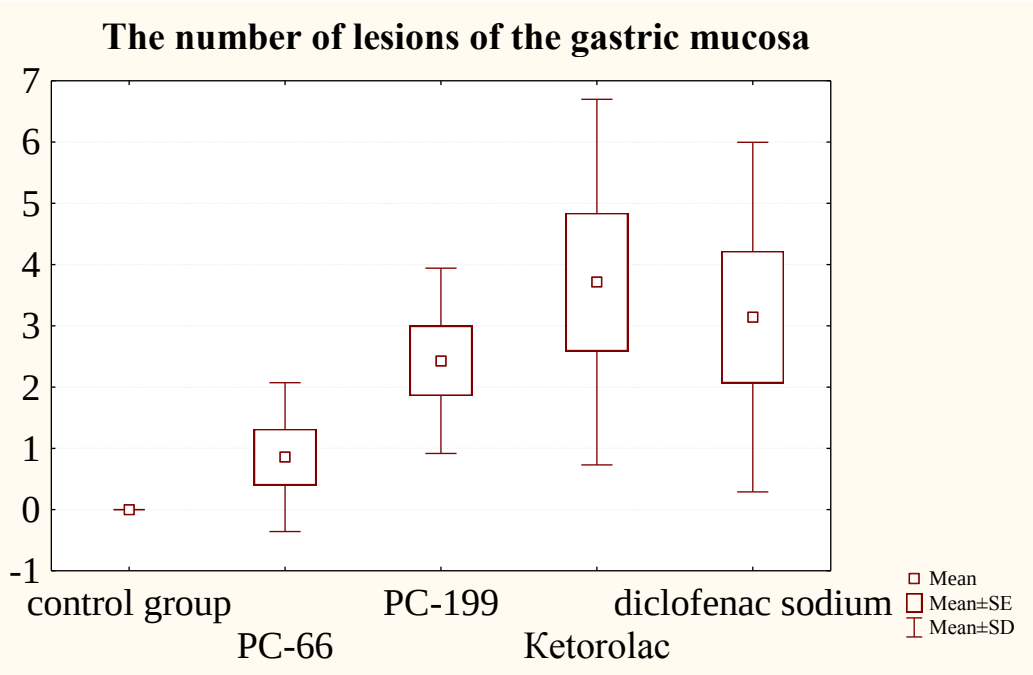
On Day 4, the spectrophotometric method revealed active prostaglandin-endoperoxide synthase (PGH) in the rats` GM homogenates against accumulation of oxidized form of adrenaline electron donor [10]. The total content of nitrites and nitrates was determined by reaction with Griess reagent (Griess test) after preliminary restoration of nitrates with a suspension of zinc dust in ammonia solution **[**11].

All values are expressed as means±s.e.mean. ANOVA and Student–Newman–Keuls test were used to determine the statistical significance of differences between groups. Differences were considered to be significant when $P<0.05$.

Results and discussions. In the study of the pharmacological effects of novel bioactive compounds with analgesic effect, the important issue is considered the evaluation of their therapeutic safety, particularly for the gastrointestinal tract (GIT), as gastric lesions are a class-specific side effect for NSAIDs [2].

Therefore, at the first phase we explored the impact of PC-66 compound and a reference medicine (diclofenac sodium) on macroscopic changes in gastric mucosa (GM) under conditions of long-term administration [12]. The results showed that administration of PC-66 compound had not presented any changes in the general condition and feeding behavior of experimental rats compared with control animals. In contrast, the rats treated with diclofenac sodium became lethargic, sedentary at the end of the experiment, yet remained reactive to external stimuli; and consumption of water and feed was not different to those from the control group.

After euthanasia, removal of stomach and its macroscopic study, it was found that 30-day administration of investigational quinazoline derivative (PC-66 compound) caused smoothing and hyperemia to the stomach mucosa (in 28.6 % of animals), minor lesions of the GM, mainly presented as punctate hemorrhages (in 71.4 % of rats). Ulcerative lesions were observed only in 42.9 % of animals, the prevalence of ulceration averaged 0.9±0.46 per 1 subject, and the severity of stomach mucosa damage was assessed 0.6±0.30 points (Fig. 1).



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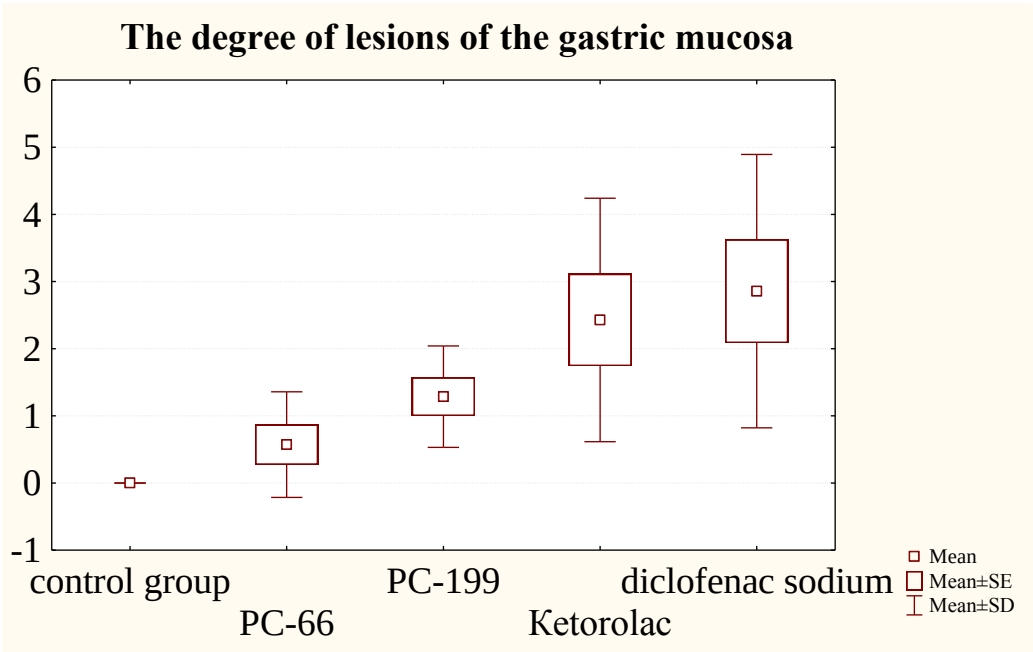

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Fig. 1. Prevalence and severity of GM lesions during a 30-day administration of PC-66 and diclofenac sodium in intact rats: *a* – prevalence; *b* – severity

All above-mentioned values were statistically significantly lower than those during administration of diclofenac sodium, which caused a stomach ulcer lesions in 71.7 % of the animals, while the average number of ulcers per 1 animal was 3.1±1.08, and the average GM lesion severity degree scored 2.9±0.77.

That means that PC-66 compound, unlike the reference medicine, did not cause significant gastric lesions under conditions of its long-term administration to intact rats.

Mechanisms of gastric stability include pre-epitelial, epithelial and post-epitelial lines of defense [13]. It is known that NSAIDs adversely affect all levels of GM protection, but their ability to cause imbalance in production of vasoconstrictive and vasodilative molecules, such as prostaglandins and nitric oxide plays the major role [14]. Therefore, at the next phase we studied the effect of PC-66 compound and diclofenac on activity of prostaglandin-H-synthase and the content of stable nitric oxide metabolites in gastric mucosa.

The data presented in Table 1 suggest that control animals had a tendency ($r>0.05$) of decreasing (15.2 %) the activity of PGH-synthetase in GM compared to intact rats during fasting.

Table 1

The activity of PGH-synthetase in supernatant GM homogenate during administration of PC-66 and diclofenac sodium

Study groups	Activity of PGH-synthetase, mmol/min/mg of protein	The trend against control, %	Level of stable NO metabolites in GM, nmol/g of tissue	The trend against control, %
Group I (control)	17.3*±1.8		310±16.4	-
Group II (PC-66)	16.1*±3.7	–6.9 %	535±19.5*	+72.6 %
Group III (diclofenac sodium)	10.7*±2.4	–38.1* %	240±14.9	–22.6 %
Group IV (intact)	20.4±3.1		300±15.2	

*Note: * – $p \leq 0.05$ compared to control*

These data correlate with the results of clinical studies, as it is known that a number of disorders in gastric mucosa prostaglandin metabolism are associated with the development of erosive defect [15]. Hunger, which may be considered a factor of stress, often contributes to erosive changes in gastric mucosa (GM). According to some data, the PGs have a protective effect on GM – they increase secretion of mucus, activate regenerative processes in the stomach mucosa, and strengthen its barrier function due to reverse diffusion of H^+ ions. In addition, PGE1 boosts formation of gastric mucosa glycoproteins, namely, N-acetylneuraminic acid and bicarbonates, thus providing GM protective properties and improving microcirculation in the gastric wall. Patients with erosive changes in gastric mucosa always presented reduced levels of PGE and PGE1 [16].

We found that during diclofenac administration, the activity of PGH-synthetase in GM significantly decreased by 38.1 % compared to the control, whilst under the influence of PC-66, the activity of this enzyme remained practically unchanged: the change was 6.9 % (Table 1).

These changes of PGH-synthetase activity in GM in case of administration of both substances may be a sign of lacking cyclooxygenase inhibitory activity of PC-66 at a dose of 1 mg/kg in contrast to diclofenac.

Study of stable NO metabolites

It is known that NO serves in humans and animals as a biological mediator in different physiologic processes in almost all organs and systems, including CNS and GIT [17]. NO is inherent of such properties as vasodilatory, antiplatelet, spasmolytic effect on smooth muscle organs (including GIT), cytoprotective, and anti-inflammatory effects [18].

It is also known that practically all NSAIDs inhibit NO synthesis in organs, which causes violation of internal organ trophism along with inhibition of vasodilation PGs production [19]. In GIT, it is accompanied by emergence of erosive and ulcerative lesions of mucosa [20]. All this has become a basis for assessment of nitric oxide stable metabolites content in the stomach lining during administration of PC-66 and diclofenac sodium.

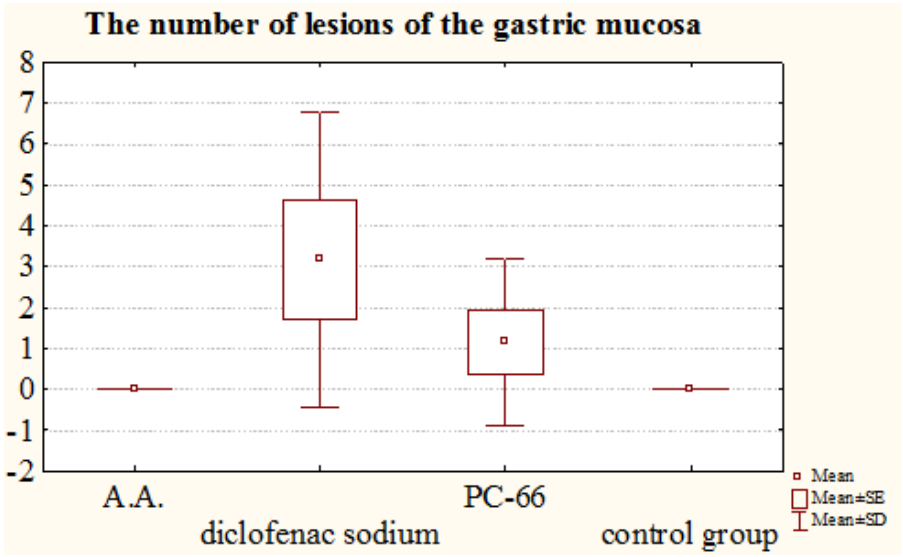
The data presented in Table 1 show no significant difference between the content of stable NO metabolites (nitrate + nitrite) in GM in the group of control (fasting+ 0.9 % NaSI) and intact animals.

Administration of PC-66 compound to rats, unlike diclofenac, was associated with a significant increase of stable NO metabolites in GM by 72.6 %, whilst this index decreased by 22.6 % ($r>0.05$) compared to control under the influence of investigational NSAID.

Based on these data, we can assume that the increase of stable nitric oxide metabolites content in GM under PC-66 at a dose of 1 mg/kg is a manifestation of its stimulating effect on production of NO in the body, that is probably one of the explanations for absence of the medicine`s ulcerogenic effect. Instead, diclofenac ability to reduce the level of stable NO metabolites may be one of the reasons for violation of GM integrity under influence of this NSAID. These data suggest greater safety of PC-66 compared to diclofenac in terms of affecting GIT.

We know that the study inflammation process may significantly amplify the pharmacological effect and toxicity of diclofenac sodium as a result of inhibitory effect of inflammation on the activity of xenobiotic-metabolizing enzymes [21]. This results in the increase of unmodified medicine content, characterized by quite significant toxicity, thus leading to more pronounced display of NSAIDs` specific toxic effects [22].

Therefore, it was necessary to establish whether the gastric safety of PC-66 compound will persist under conditions of experimental inflammation (adjuvant arthritis). The results suggest that almost half of the non-treated animals (42.9 %) with adjuvant arthritis at the end of treatment period presented signs of minor damage to the integrity of GM, including smoothed mucosa, hyperemia and isolated punctate hemorrhage, corresponding to lesion severity of average 0.7±0.36 points. No evidence of ulceration was found (Fig. 2).



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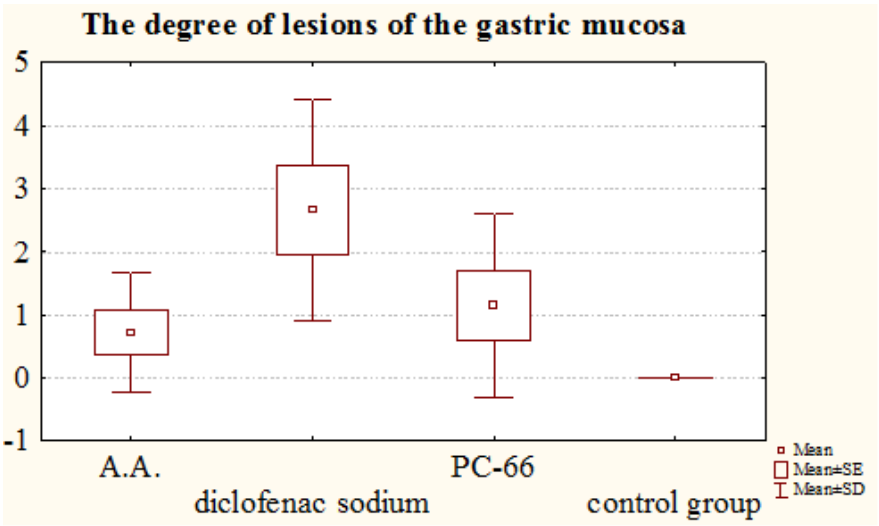

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Fig. 2. Prevalence and severity of GM lesions during a 30-day administration of PC-66 and diclofenac sodium in rats with AA: *a* – prevalence; *b* – severity

Treatment of rats with diclofenac sodium caused significant adverse changes in the GM: all rats were found smoothened mucosa, hyperemia, hemorrhage, which reached an average of 1.7–2.0 points. Multiplicity of ulceration in this group was 3.7±1.36 per 1 animal and severity of lesions – 3.0±0.69 points. One animal died due to perforated ulcer and peritonitis. Unlike the reference medicine, administration of PC-66 compound to rats did not cause death to animals; the degree of surface lesions was not statistically different from the control (untreated AA subjects), and the average number of ulcers and GM lesion severity were statistically lower compared to diclofenac sodium administration and made 1.1±0.77 and 1.1±0.55 ($p<0,05$), respectively.

7. Findings from the research and prospects of further development of this area

Thus, our findings clearly show that the new derivate of 4- [4-oxo-4H-quinazoline-3-yl] benzoic acid (PC-66) has no damaging effect on the gastric mucosa both in intact animals and in rats with experimental inflammation. The basis of its GM safety rests on lacking inhibitory effect on production of vasodilator molecules in GM of PC-66 compound, unlike diclofenac sodium. Further studies of action mechanisms of derivate of 4- [4-oxo-4H-quinazoline-3-yl] benzoic acid will make possible to justify the feasibility of development of a medicine for treatment of acute and chronic pain syndromes on the basis of the study medication.

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Kramar Hanna, assistant, Department of Pharmacy, National Pirogov Memorial Medical University; Pirogova str., 56, Vinnitsa, Ukraine, 21000
E-mail: annachivanna@gmail.com