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Investigation of ulcerogenic property of new arylpropionic derivative of non protein amino acid

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Development of new agents among nonsteroidal anti-inflammatory drugs (NSAIDs) remains an important field in drug discovery, as in spite of their widely accepted analgesic, anti-inflammatory and antifever properties, making them most commonly used drugs worldwide, they possess a wide range of side effects [13]. Approximately 30% of hospitalizations of adverse drug reactions are caused by NSAIDs [10]. Adverse reactions can affect various systems, such as cardiovascular system, kidneys, and especially gastrointestinal (GI) tract, and sometimes can cause fatal reactions (reported peptic ulcer bleeding related mortality rates range from 3,4% to 14% in developed countries) [3].

The mechanisms responsible for NSAID-induced ulcerative lesions of the GI tract are not yet completely understood. It is well known that NSAIDs injure the gut by systemic GI tract toxicity associated with inhibition of mucosal prostaglandin synthesis derived from COX inhibition, local irritation of the mucosa and platelet inhibition, also considered to be a key mechanism of GI tract bleeding lesions [7].

NSAIDs possess systemic ulcerogenic activity due to inhibition of cyclooxygenase (COX) enzyme – the key enzyme in prostaglandin (PG) synthetic process, which is not only a mediator of inflammation, but plays a very important essential homeostatic role in cytoprotection of gastric mucosa, hemostasis, renal function, gestation and parturition [9,15,18].

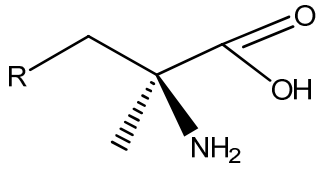
COX enzyme has three isoforms. COX-1 is a constitutive enzyme, expressed in most tissues; it serves as a housekeeping enzyme responsible for normal cell homeostasis. COX-2 expression is inducible with inflammation but in a limited number of organs it is expressed in a normal physiologic condition as well [11] and COX-3 is expressed mainly in CNS [2]. Thus, PGs are ubiquitous compounds that mediate a variety of physiologic and pathologic processes, and most of the systemic adverse effects of NSAIDs are a direct result of their mechanism of action [6]. Moreover, it was demonstrated, that depression of mucoprotective prostaglandins synthesis, caused by inhibition of

constitutively expressed COX-1, increases sensitivity of gastric and duodenal mucosa, becomes more to luminal acid and pepsin [16]. Direct contact with gastric mucosa and its damage through oral dose is accompanied by pH changing. GI tract complication (bleedings) could be worsened by disbalance between prostacycline and tromboxan, caused by inhibition of COX-1 [8].

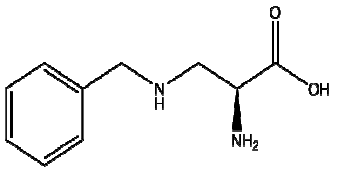
Based on the above data, temporary masking the acidic group of NSAIDs to decrease the GI toxicity due to direct injury has been postulated as one of the promising ways for development of new NSAIDs with possible low rate of the mentioned side effects [2, 9].

In view of this we have been searching for new potential sources among arylpropionic acid derivatives from non protein amino acids (NPAA). Our choice was based on one hand on the structure – activity relationship (SAR) of NSAIDs, postulating that functional groups important for COX inhibition activity are the aromatic ring, carboxyl group, and the distance between these two groups, corresponding to the distance equal to two carbon atoms. On the other hand, NPAAs have quite a wide spectrum of pharmacological actions, such as anti-inflammatory, anticancer, antibacterial activities [5,14].

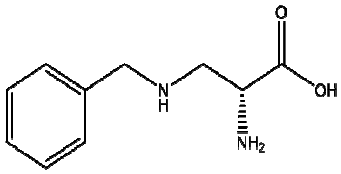
Novel non protein amino acids – derivatives of 2-amino-3-propionic acid were synthesized in the Institute of Biotechnology NASRA with the illustrated below common structure:



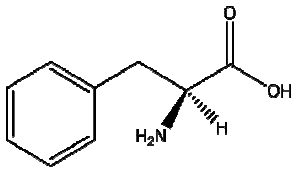
In the previous *in vitro* investigation there were observed 4 derivatives for COX inhibiting activity:



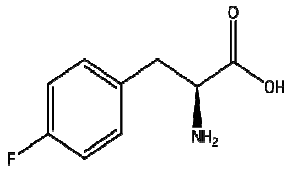
S(+)-2-amino-3-(benzylamino)propanoic acid
NPAA34



R(-)-2-amino-3-(benzylamino)propanoic acid
NPAA35



S(-)-2-amino-2-methyl3-phenylpropanoic acid
NPAA36



S(-)-2-amino-3-(4'-fluoro)phenylpropanoic acid
NPAA36

It was found out that these structures possess both COX-1 and COX-2 inhibitor activities and the more potent one was NPAA-36. The following *in vivo* experiments confirmed these data and proved their anti-inflammatory and antinociceptive activities [1,19].

The aim of the presented study was investigation of ulcerogenic properties of NPAA-36 in comparison with Ketoprofen for further its developing as a new potential NSAID.

Materials and Methods

Assessment of the ulcerogenic properties of NPAA 36 was carried out on the experimental model of ulcerogenicity [17]. Before testing, the animals were kept in standard laboratory vivarium conditions. The experiment was performed on 40 adult male albino rats, weighing 180-200 g., divided into 5 groups, 8 rats in each. All experimental manipulations with animals were performed according *the PHS Guide for the Care and Use* of *Laboratory Animals* (1996). Rats were fasted for 18 hrs prior to the experiment. Animals were stored in specially designed containers, which contained a metal net between the placed rats and the cage floor, to avoid coprophagy. The first group of rats, which was also considered as a control group, received only 0.5 ml of distilled water once a day for 2 days. The rats of the second and third groups were administered 0.5 ml solutions of Ketoprofen and NPAA-36 in dose 50 mg/kg accordingly. The rats of the fourth and fifth groups received 0.5 ml solution of Ketoprofen and NPAA-36 in dose 100 mg/kg. All investigated compounds were administered orally by special oral lavage needle that provides the constant dose. 7 hrs. after the last dose, all rats were sacrificed. The rats' stomachs were excised, opened along big curvature and washed with 0.9% sodium chloride solution, then fixed on filter paper and maximally smoothed out. The observed stomachs were filmed by a digital camera with a ruler. The degrees of stomach mucosa damage caused by Ketoprofen and NPAA-36 were calculated using the Image J program, which allows to evaluate ratio of total and summary damaged surfaces of the stomach by expressing using the ruler as a measuring unit pointer for extrapolation of appropriate pixels to mm^2 square.

Results and Discussion

The presence of lesions following oral administration of NPAA 36 as well as Ketoprofen has been taken as an evidence for their ulcerogenic effects. As evident the conducted experiments, there are no changes in stomach mucosa of control group rats receiving only distilled water. Gross visual observation of rat stomachs indicated a gastric mucosal injury and several hemorrhagic steaks in animals treated with Ketoprofen. The results of experimental data extrapolated by Image programme have shown that the surface of stomach

mucosa damage of animals which had received Ketoprofen in dose 50 mg/kg once a day during two days was 15,87 ± 5,77 mm^2, which is equal to 2,06 ± 0,569% of total surface (761,19 ± 133,35 mm^2). Ketoprofen in dose 100 mg/kg in the same regimen resulted in disturbance of rats' stomach mucosa completeness for 14,65 ± 6,24 mm^2, which is equal to 2,18 ± 0, 92% of 675,84 ± 79,1 mm^2 of total stomach surface. As compared to Ketoprofen, the NPAA-36 administered in both 50 mg/kg and 100 mg/kg doses did not induce any essential changes compared to the stomach mucosa of the control group rats, only in some rare cases there was observed a week redness.

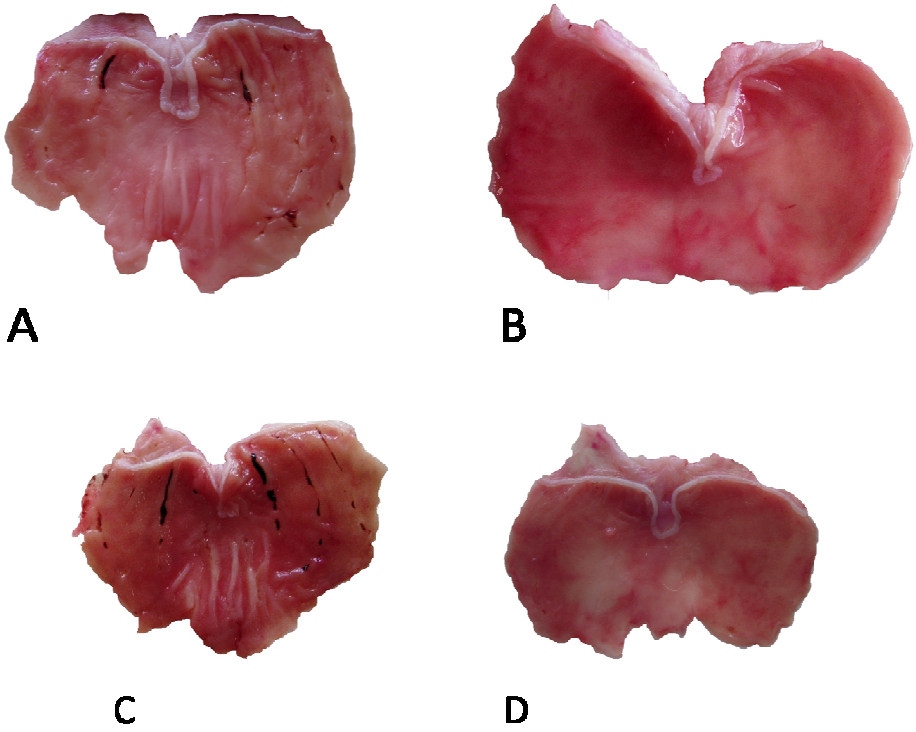


Figure. Rats' stomach mucosa surface: A – rats treated by Ketoprofen in 50 mg/kg dose, B – rats treated by NPAA in 36,50 mg/kg dose; C – rats treated by Ketoprofen in 100 mg/kg dose; D – rats treated by NPAA 36,50 in mg/kg dose.

Thus it has been demonstrated, that the revealed anti-inflammatory and antinociceptive effects of S(-)-2-amino-2-methyl-3-phenylpropionic acid (NPAA 36), in comparison with Ketoprofen, are not accompanied by any essential visible changes of stomach mucosa surface even in doses 50 and 100 mg/kg. The mentioned differences of gastrointestinal action of the investigated amino acid are probably due to the presence of additional amine group insertion in its structure, masking the acidic properties and leading to decrease of the gastrointestinal toxicity through the falling of pH, as it has been postulated. These data indicate that the new described derivative of NPAA could be a

potential source for development of new anti-inflammatory drugs with lower GI toxicity.

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Исследование ульцерогенной способности нового производного арилпропионовой кислоты в ряду небелковых аминокислот

С. А. Григорян

С целью разработки новых нестероидных противовоспалительных средств (НПВС) с возможно слабым проявлением побочных эффектов исследована способность язвообразования S (-)-2-амино-2-метил-3- фенилпропионовой кислоты (NPAA 36) – производного арилпропионовой кислоты небелковых аминокислот (NPAA) с ЦОГ ингибирующей активностью. Оценка способности язвообразования исследуемого производного по сравнению с кетопрофеном проводилась в экспериментальной модели язвообразования. Полученные результаты свидетельствуют о том, что кетопрофен в дозах 50 мг/кг и 100 мг/кг вызывает покраснение и кровоизлияния, нарушая целостность слизистой желудка на поверхности, составляющей соответственно 2,06 ± 0,569% и 2,18 ± 0,9% от общей поверхности желудка. В отличие от кетопрофена, введение NPAA-36 в обеих дозах не вызывало каких-либо существенных изменений слизистой желудка по сравнению с теми же данными контрольной группы животных. Полученные данные свидетельствуют, что изученное производное NPAA может служить потенциальным источником для разработки новых противовоспалительных препаратов с возможно низкой вероятностью побочных эффектов на ЖКТ.

Ոչ սպիտակուցային ամինաթթուների շարքին պատկանող արիլպրոպիոնաթթվի ածանցյալի խոցածին հատկության հայտնաբերումը

Ս. Հ. Գրիգորյան

Պոտենցիալ նոր ոչ ստերոիդային հակաբորբոքային դեղերի (ՈՍՀԲԴ) ստեղծման նպատակով ուսումնասիրվել է ոչ սպիտակուցային ամինաթթուների շարքին պատկանող արիլպրոպիոնաթթվի ածանցյալ հանդիսացող ցիկլոօքսիգենազա ֆերմենտը (ՑՕԳ) ընկճող ակտիվությամբ, հակաբորբոքային և հականոցիցեպտիվ հատկություններով օժտված S(-)-2-ամինո-2-մեթիլ-3-ֆենիլպրոպանաթթվի (NPAA 36) խոցածին հատկությունը: Նշված միացության խոցածին հատկու-

թյունը գնահատվել է խոցագոյացման փորձարարական մոդելում և համեմատվել կետոպրոֆենի հետ: Ստացված արդյունքները վկայում են, որ կետոպրոֆենը 50մգ/կգ և 100 մգ/կգ դեղաչափով՝ առաջացնելով ստամոքսի լորձաթաղանթի կարմրություն և արյունազեղումներ, խախտում է ատամոքսի մակերեսի ամբողջականությունը 2,06 ± 0,569% և 2,18 ± 0,92% համապատասխանաբար: Ի տարբերություն կետոպրոֆենի առաջացրած փոփոխությունների, NPAA 36-ի ներմուծումը երկու դեղաչափերով էլ չի ուղեկցվում որևէ էական փոփոխություններով՝ ստուգիչ խմբի կենդանիների ստամոքսի լորձաթաղանթի կառուցվածքի համեմատ: Այս տվյալները մատնանշում են, որ նկարագրված ոչ սպիտակուցային ամինաթթուն կարող է ծառայել որպես խոստումնալից աղբյուր նոր հակաբորբոքային դեղերի ստեղծման համար, որոնք կցուցաբերեն հնարավորինս նվազ ՍԱՀ թունայնություն:

References

1. *Grigoryan S. et al*. The investigation of antiinflammatory activity of some novel synthetic α-non-protein amino acids. The New Armenian Medical Journal, 2010, Vol.4, I, p. 52-53.
2. *Botting R., Ayoub S.S.* COX–3 and the mechanism of action of paracetamol/acetaminophen. Prostaglandins Leukot. Essent. Fatty Acids, 2005 Feb;72(2):85-7.
3. *Budimir I., Stojsavljevic S., Nikolic M. et al.* Open Bleeding Peptic Ulcer: Epidemiology, Treatment and Prognosis. J. Gastroenterol., 2017; 3(1): 1007.
4. *Bundgaard H. & Nielsen N.M.* New trends in pharmacokinetics. Int. J. Pharm., 1988; 43:101.
5. *Cheng H. et al*. Control of cyclooxygenase – 2 expression and tumorigenesis by endogenous 5 – methoxytryptophan. PNAS, 2012,Vol. 109 (33), p. 13231 - 13236.
6. *Inger L. Meek*. Non-steroidal Anti-inflammatory Drugs: An Overview of Cardiovascular Risks. Pharmaceuticals (Basel), 2010 Jul; 3(7): 2146–2162.
7. *Lene Mellemkjær et al.* Upper gastrointestinal bleeding among users of NSAIDs: a population-based cohort study in Denmark. Br. J. Clin. Pharmacol., 2002 Feb; 53(2): 173–181.
8. *Marjan Mokhtare et al.* Lower Gastrointestinal Bleeding Due to Non-Steroid Anti-Inflammatory Drug-Induced Colopathy. Case Report and Literature Review. Middle East J. Dig. Dis., 2013 Apr; 5(2): 107–111.
9. *Narumiya S., Fitzgerald G.A.* Genetic and pharmacological analysis of prostanoid receptor function. J. Clin. Invest., 2001; 108: 25-30.
10. *Patompong Ungprasert et al*. What Is the “Safest” Non-Steroidal Anti-Inflammatory Drugs? American Medical Journal, 2012, 3 (2): 115-123.
11. *Rouzer C.A., Marnett L.J*. Cyclooxygenases: structural and functional insights. Journal of Lipid Research, J. Lipid. Res., 2009 Apr; 50 Suppl: S29-34.
12. *Sahu Jagdish K.* Evaluation of ulcerogenicity of Ketoprofen glucopyranoside derivatives, IJRAP 2010, 1 (1): 186-191.
13. *Scarpignato C., Hunt R.H*. Nonsteroidal anti-inflammatory drug–related injury to the gastrointestinal tract: clinical picture, pathogenesis, and prevention. Gastroenterol. ClinNorth Am., 2010 Sep;39(3):433-64.
14. *Schenk S.U. et al*. Broad antifungal activity of β – isoxazolinonyl – alanine, a non protein amino acid from roots of pea (Pisum sativum L.) seedlings. Biology and Fertility of Soil, 1991,Vol.11(3), p.1062 – 1064.

15. *Smith W.L., Marnett L.J., DeWitt D.L.* Prostaglandin and thromboxane biosynthesis. Pharmacol. Ther., 1991; 49:153-179.
16. *Tanigawa T. et al.* Rebamipide, a mucoprotective drug, inhibits NSAIDs induced gastric mucosal injury: possible involvement of the downregulation of 15-hydroxyprostaglandin dehydrogenase. J. Clin. Biochem. Nutr., 2011 Mar;48(2):149-53.
17. *Tous S., Fathy M., Fetih G., Sheryhan F.* Preparation and Evaluation of Ketoprofen-loaded Calcium alginate beads. International Journal of PharmTech Research, 2014, Vol.6, 3, p.1100-1112
18. *Vane J.R. et al*. Cyclooxygenases 1 and 2. Ann. Rev. Pharmacol. Toxicol., 1998; 38: 97-120.
19. *Zhamharyan A.G. et al*. Antinociceptive activity of 2 – amino – 3 – arylpropionic acid derivatives. PhysioMedi., Vol. 2, 2013, p. 188 – 192.