DE GRUYTER OPEN

DOI: 10.1515/folmed-2017-0095

ORIGINAL ARTICLE, MEDICINE

In Vivo Evaluation of Anti-inflammatory Activity of 2-[3-Acetyl-5-(4-chloro-phenyl)-2-methyl-pyrrol-1-yl]-4-methylsulfanyl-butyric Acid

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Received: 26 Jan 2017
Accepted: 02 Oct 2017
Published Online: 09 Oct 2017
Published: 30 June 2018

Key words: N-pyrrolylcarboxylic acid, NSAIDs, coxibs, inflammation, carrageenan-induced paw edema

Citation: Zlatanova H, Vladimirova S, Kostadinov I, Bijev AT. In vivo evaluation of anti-inflammatory activity of 2-[3-Acetyl-5-(4-chloro-phenyl)-2-methyl-pyrrol-1-yl]-4-methylsulfanyl-butyric acid. Folia Med (Plovdiv) 2018;60(2): 270-4.

doi: 10.1515/folmed-2017-0095

Background: Persisting inflammatory stimuli cause chronic inflammation recognized as the major factor contributing to the development of a number of diseases. One group of drugs used in the treatment of chronic inflammation is the group of non-steroidal anti-inflammatory drugs and, more specifically, the selective COX-2 inhibitors (coxibs). However, most of the coxibs were withdrawn from the market in view of their safety profile. In the present study, 2-[3-Acetyl-5-(4-chloro-phenyl)-2-methyl-pyrrol-1-yl]-4-methylsulfanyl-butyric acid (compound 3e), an N-pyrrolylcarboxylic acid derivative structurally related to celecoxib, is evaluated for anti-inflammatory activity after single and multiple (14 days) administration using an animal inflammation model.

Aim: To evaluate the anti-inflammatory properties of 2-[3-Acetyl-5-(4-chloro-phenyl)-2-methyl-pyrrol-1-yl]-4-methylsulfanyl-butyric acid (compound 3e) after single and multiple (14 days) administration using an animal inflammation model.

Materials and methods: Forty Wistar rats were allocated into 5 groups (n=8) treated with saline (controls), diclofenac (25 mg/kg b.w.), compound 3e (10, 20 and 40 mg/kg b.w.) intraperitoneally. The volume of the right hind paw of the animals of all groups is measured prior to treatment and two, three and four hours after administration of carrageenan using a plethysmometer (Ugo Basile, Italy). The percentage of paw edema is calculated using the Trinus formula.

Results: In a single administration, compound 3e in doses of 10 and 20 mg/kg b.w. did not inhibit paw edema, while a dose of 40 mg/kg b.w. significantly inhibited carrageenan-induced paw edema at 2 hours in comparison with the control group. After continuous administration, compound 3e in doses of 10, 20 and 40 mg/kg b.w. significantly reduced paw edema at 2, 3, and 4 hours compared to animals treated with saline.

Conclusions: Compound 3e shows anti-inflammatory properties similar to those of diclofenac after continuous administration.

BACKGROUND

Inflammation response is an answer to noxious stimuli.[1] Acute inflammation is a self-limiting process that usually restores tissue homeostasis. Persisting inflammatory stimuli or deregulation of the resolution phase mechanisms cause chronic inflammation, which is recognized as a major risk factor for the development of a number of diseases including atherosclerosis, arthritis, and cancer.[2]

One group of drugs used in the treatment of chronic inflammatory diseases are non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs are among the most often prescribed drugs.[3] They are the fourth most commonly sold drugs in the global pharmaceutical market after antibiotics, cardiovascular and psychotropic medications. After the discovery of their mechanism of action (inhibition of the cyclooxygenase (COX)) and the identification of two COX isoenzymes, selective COX-2 inhibitors (coxibs) were introduced for the purpose of better gastrointestinal tolerance. However, most of them were withdrawn from the market in view of their safety profile.[4-6] This necessitates a search for new drug medications with analgesic and anti-

inflammatory effects.

2-[3-Acetyl-5-(4-chloro-phenyl)-2-methyl-pyrrol-1-yl]-4-methylsulfanyl-butyric acid (compound 3e) is a newly synthesized N-pyrrolylcarboxylic acid derivative. The chemistry, design, synthesis and characterization by spectroscopy and TLC (thin line chromatography) were described by Vladimirova et al.[7]

The pyrrolic ring is chosen as a central core due to the involvement of this heterocycle in the pharmacophore system of a large number of classic and contemporary NSAIDs.[8-10] The structure of the target molecule is oriented to the architecture of the selected prototype for a contemporary anti-inflammatory agent celecoxib (a selective COX-2 inhibitor), thus expecting manifestation of its characteristic pharmacological activity.

The carrageenan model chosen in this study is generally used to assess potential anti-inflammatory activity of novel substances. The inflammation induced by carrageenan possesses two distinct phases[11], with prostaglandins playing a major role[12]. This makes the model particularly suitable for registering anti-inflammatory effect of compounds with suspected COX-blocking mechanism of action.

The aim of the present study was to evaluate the anti-inflammatory properties of 2-[3-Acetyl-5-(4-chloro-phenyl)-2-methyl-pyrrol-1-yl]-4-methylsulfanyl-butyric acid (compound 3e) after single and multiple (14 days) administration using an animal inflammation model.

MATERIALS AND METHODS

The experimental setting was approved by the Ethics Committee on Animals of the Bulgarian Food Safety Agency (license No 128/09.12.2015) and by the Ethics Committee at the Medical University of Plovdiv, (protocol No 2/31.03.2016).

Experimental animals

Forty adult male Wistar rats weighing 150±20 grams were included in the study. They were randomly allocated into 5 parallel experimental groups with 8 animals per group as follows:

Group I – a control group, treated intraperitoneally (i.p.) with saline;

Group II – positive controls, treated with the referent anti-inflammatory drug diclofenac (25 mg/kg b.w., i.p.);

Group III – treated with compound 3e in a dose of 10 mg/kg b.w. (i.p.);

Group IV – treated with compound 3e in a dose of 20 mg/kg b.w. (i.p.);

Group V – treated with compound 3e in a dose of 40 mg/kg b.w. (i.p.).

All substances were dissolved in saline. The drugs were administered intraperitoneally with the doses expressed as milligram salt per kilogram body weight (mg/kg b.w.). The doses used in the experiment were determined according to acute oral toxicity tests. The animals were maintained on a light-dark cycle of 12:12 h in a temperature-controlled environment with food and water available ad libitum.

Carrageenan-induced paw edema.

The volume of the right hind paw of the animals of all groups was measured prior to treatment using a plethysmometer (Ugo Basile, Italy). 0.1 ml of a 1% solution of carrageenan in 0.9% NaCl was injected in the right hind paw of all animals to induce edema. Immediately after injection of carrageenan, the control group was given saline solution intraperitoneally; the positive control group received diclofenac and the animals of groups III, IV and V - compound 3e. The volume of displaced liquid from the right hind paw of the rats was measured using a plethysmometer two, three and four hours after administration of carrageenan. The percentage of paw edema was calculated using the Trinus formula.

Statistical analysis of the results was performed with IBM SPSS 20.0 software. The Kolmogorov-Smirnov test was used to determine the normality of distribution. Arithmetic mean and standard error of the mean (± SEM) were determined for each indicator. Comparison of the results between groups was performed using the Independent Sample t-test. A p value of < 0.05 was considered statistically significant.

RESULTS

The reference anti-inflammatory drug diclofenac showed significant reduction in paw edema at all hours after both single and multiple administration.

After a single administration, the compound 3e in doses of 10 and 20 mg/kg b.w. did not inhibit paw edema compared to the animals treated with saline solution. A dose of 40 mg/kg b.w. significantly inhibited the carrageenan-induced paw edema at 2 hours in comparison with the control group. After multiple administration (14 days), compound 3e in doses of 10, 20, and 40 mg/kg b.w. significantly reduced paw edema at 2, 3, and 4 hours compared to animals from the control group.

Table 1. Comparison between the controls and the groups treated with diclofenac and compound 3e in doses of 10, 20 and 40 mg/kg b.w. in carrageenan-induced paw edema test

Group	Hours	mean±SEM	t	p	$mean_1$±SEM_1	t_1	p_1
Controls	2	47.08±4.06	-	-	45.77±3.79	-	-
	3	35.70±6.88			43.67±3.88		
	4	41.55±3.75			49.24±4.33		
Diclofenac	2	21.86±2.33	5.57	<0.001*	14.46±0.89	8.05	<0.001*
	3	14.41±2.93	2.98	0.011*	11.19±1.39	7.88	<0.001*
	4	16.54±3.02	5.25	<0.001*	7.70±1.25	9.21	<0.001*
3e (10 mg/kg)	2	40.71±6.65	0.79	0.44	25.75±5.56	2.97	0.01*
	3	40.36±4.91	0.56	0.58	20.61±4.37	3.95	0.001*
	4	46.23±4.15	0.83	0.42	12.76±2.84	7.04	<0.001*
3e (20 mg/kg)	2	34.86±6.05	1.63	0.13	10.01±2.28	8.09	<0.001*
	3	40.41±4.99	0.56	0.58	10.92±3.86	5.98	<0.001*
	4	41.56±6.93	0.00 1	1.00	9.76±4.19	6.55	<0.001*
3e (40 mg/)kg	2	25.43±3.59	4.01	0.001*	10.93±3.72	6.56	<0.001*
	3	26.45±4.31	1.17	0.26	10.22±1.78	7.83	<0.001*
	4	30.78±3.88	1.98	0.07	12.64±4.01	6.20	<0.001*

mean±SEM, t, p - after single administration;
$mean_1$±SEM_1, t1, p1 - after multiple (14 days) administration; *p < 0.05

The specific values of paw edema in percentages, as well as the values of t-criterion and p-value are presented in **Table 1**.

DISCUSSION

The results in our study suggest that in experimental conditions 2-[3-Acetyl-5-(4-chloro-phenyl)-2-methyl-pyrrol-1-yl]-4-methylsulfanyl-butyric acid has anti-inflammatory activity in the carrageenan-induced inflammation model. This effect is significant after continuous administration of the compound.

The experimental edema induced by carrageenan administration in rats and the acute exudative inflammation in humans possess huge similarities in vascular and cell reactions. Histamine, serotonin and several pro-inflammatory cytokines (TNF-α, IL-1β) are involved in the pathogenesis of the carrageenan-induced swelling during the initial vascular phase; afterwards bradykinin mediates increased vascular permeability and prostaglandins participate in the acute inflammatory reaction during the cellular phase (3-5 hours).[13] Suppression of this last phase is typical of NSAIDs and correlates to a big extent with their therapeutic efficacy.[13] Compound 3e after multiple administration significantly inhibits paw swelling at 2, 3, and 4 hours, with the most pronounced effect at 4 hours. That would be in line with the expected of this substance cyclooxygenase inhibiting activity. Since the tested substance also inhibits paw edema at the early hours, we can hypothesize that it possesses antagonistic action to all mediators involved in the vascular and cellular phases.

After single administration, compound 3e in a dose 40 mg/kg b.w. inhibits paw swelling at 2 hours and nearly reaches significance at 4 hours. We could speculate that higher doses are necessary for blocking COX effectively. This could hint at a competitive inhibition mechanism and at possible linear kinetics and graded dose-response relationship. After continuous administration the registered anti-inflammatory effect is significant and persistent even at dose of 10 mg/kg b.w., which could indicate that accumulation of the compound is necessary for stable binding with COX at low doses. Since the

registered values of edema inhibition of doses 20 and 40 mg/kg b.w. are approximately the same, we could theorize that after multiple administration and probable accumulation of the compound, non-linear kinetics are observed.

In similar studies of novel pyrrolic compounds, Bijev et al. and Lessigiarska et al. found that the compounds significantly inhibited paw edema, showing anti-inflammatory effect greater even than that of indomethacin which was used as a reference drug.[14-16] This confirms the significance of the pyrrolic heterocycle for the anti-inflammatory effect shown by compound 3e.

Further in vitro studies of COX-1/COX-2 activity and selectivity of the compound are necessary to establish the specific mechanisms of anti-inflammatory action of compound 3e.

CONCLUSIONS

Compound 3e - (2-[3-Acetyl-5-(4-chloro-phenyl)-2-methyl-pyrrol-1-yl]-4-methylsulfanyl-butyric acid) shows significant anti-inflammatory activity, inhibiting both vascular and cellular phases of inflammation after multiple (14 days) administration in rats. The anti-inflammatory effect is similar to the one induced by diclofenac, which suggests suitability of the compound as a potential novel anti-inflammatory drug.

ACKNOWLEDGEMENTS

This research was financially supported by a Research grant from Medical University - Plovdiv (NO-7/2015).

REFERENCES

1. Maddila S, Gorle S, Sampath C, et al. Synthesis and anti-inflammatory activity of some new 1,3,4-thiadiazoles containing pyrazole and pyrrole nucleus. J Saudi Chem Soc 2016; 20,Suppl.1:306-12.
2. Wallace JL, Ianaro A, Flannigan KL, et al. Gaseous mediators in resolution of inflammation. Semin Immunol 2015;27(3):227-33.
3. Lee I, Cryer B. Epidemiology and role of nonsteroidal anti-inflammatory drugs in causing gastrointestinal bleeding. Gastrointest Endoscopy Clin N Am 2011;11:597-612.
4. Singh BK, Haque SE, Pillai KK. Assessment of nonsteroidal anti-inflammatory drug-induced cardiotoxicity. Expert Opin Drug Metab Toxicol 2014; 10(2):143-56.
5. Macías Á, Moreno C, Moral-Sanz J, et al. Celecoxib blocks cardiac Kv1.5, Kv4.3 and Kv7.1 (KCNQ1) channels. Effects on cardiac action potentials. J Mol Cell Cardiol 2010;49:984-92.
6. Anwar A, Anwar IJ, Delafontaine P. Elevation of cardiovascular risk by non-steroidal anti-inflammatory drugs. Trends Cardiovasc Med 2015;25(8):726-35.
7. Vladimirova S, Bijev A. An access to new N-pyrrolylcarboxylic acids as potential COX-2 inhibitors via Paal-Knorr cyclization. Heterocycl Commun 2014;20(2):111-5.
8. Danchev N, Bijev A, Yaneva D, et al. Synthesis, acute toxicity and analgesic activity of new derivatives of pyrrole. Arch Pharm 2006;339:670-4.
9. Bocheva A, Bijev A, Nankov A. Further evaluation of series anti-inflammatory N-pyrrolylcarboxylic acids: effects on the nociception in rats. Arch Pharm 2006;339(3):141-4.
10. Mohamed MS, Fathallah SS. Pyrroles and fused pyrroles: synthesis and therapeutic activities. Mini Rev Org Chem 2014;11(4):477-507.
11. Kostadinov I, Delev D, Petrova A, et al. Study on anti-inflammatory and immunomodulatory effects of clomipramine in carrageenan- and lipopolysaccharide-induced rat models of inflammation. Biotechnol Biotechnol Equip 2014;28(3):552-8.
12. Meshram GG, Kumar A, Rizvi W, et al. Evaluation of the anti-inflammatory activity of the aqueous and ethanolic extracts of the leaves of Albizzia lebbeck in rats. J Tradit Complement Med 2016;6:172-5.
13. Khedir SB, Mzid M, Bardaa S, et al. In vivo evaluation of the anti-inflammatory effect of Pistacia lentiscus fruit oil and its effects on oxidative stress. Evid Based Compl Alt Med 2016;2016:6108203.
14. Lessigiarska I, Nankov A, Bocheva A, et al. 3D-QSAR and preliminary evaluation of anti-inflammatory activity of series of N-pyrrolylcarbocylic acids. Farmaco 2005;60(3):209-18.
15. Bijev A, Yaneva D, Bocheva A, et al. Ligand based design, synthesis and primary in vivo screening of pyrrole derivatives as potential tricyclic anti-inflammatory agents. Drug Res 2006;11:753.
16. Bijev A, Yaneva D, Vladimirova S. Synthesis of new pyrrole derivatives as potential nonsteroidal anti-inflammatory agents. Ann Univ Sofia, Fac Chemie 2004;97:263-8.

Ин виво оценка противовоспалительной активности 2- [3-ацетил-5- (4-хлорфенил) -2-метилпиррол-1-ил] -4-метил сульфанил масляной кислоты

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Дата получения: 26 января 2017
Дата приемки: 02 октября 2017
Дата онлайн публикации: 09 октября 2017
Дата публикации: 30 июня 2018

Ключевые слова: N-пирролилкарбоновая кислота, НПВП, коксибы, воспаление, отек лапы, индуцированный каррагенаном

Образец цитирования:
Zlatanova H, Vladimirova S, Kostadinov I, Bijev AT. In vivo evaluation of anti-inflammatory activity of 2-[3-Acetyl-5-(4-chlorophenyl)-2-methyl-pyrrol-1-yl]-4-methylsulfanyl-butyric acid. Folia Med (Plovdiv) 2018;60(2):270-4.
doi: 10.1515/folmed-2017-0095

Введение: Персистентные воспалительные стимулы вызывают хроническое воспаление, признанное в качестве основного фактора, способствующего развитию ряда заболеваний. Одной из групп лекарств, используемых при лечении хронического воспаления, являются нестероидные противовоспалительные препараты и, более конкретно, селективные ингибиторы COX-2 (коксибы). Однако большинство из них были выведены с рынка с учетом их профиля безопасности. В нашем исследовании 2- [3-ацетил-5- (4-хлорфенил) -2-метилпиррол-1-ил] - 4-метил сульфанил масляной кислоты (соединение 3e), производное N-пирролилкарбоновой кислоты структурно связанной с целекоксибом, исследуются на предмет противовоспалительной активности после однократного и многократного (в течение 14 дней) введения с использованием модели воспаления животных.

Цель: Проведение оценки противовоспалительных свойств 2- [3-ацетил-5- (4-хлорфенил) -2-метилпиррол-1-ил] - 4-метил сульфанил масляной кислоты (соединение 3e) после однократного и многократного (в течение 14 дней) введение с использованием модели воспаления животных.

Материалы и методы: 40 крыс вида Вистар были распределены в 5 групп (n = 8), обработанных физиологическим раствором (контрольная группа), диклофенаком (25 мг / кг в.т.), соединением 3e (10, 20 и 40 мг / кг в.т.) внутрибрюшинно. Объём правой задней лапы у животных всех групп измеряется до начала лечения и через два, три и четыре часа после введения каррагинана с использованием плетизмометра (Уго Басил, Италия). Процент отека лапы вычисляется с использованием формулы Тринуса.

Результаты: При единичном введении соединение 3e в дозах 10 и 20 мг / кг в.т. не ингибирует отечность лапы, в то время как дозы 40 мг / кг в.т. значительно подавляли вызванную каррагином отечность лапы через 2 часа по сравнению с контрольной группой. После непрерывного введения соединения 3e в дозах 10, 20 и 40 мг / кг в.т. значительно уменьшилась отечность лапы через 2, 3 и 4 часа по сравнению с животными, получавшими физиологический раствор.

Заключение: Соединение 3e показывает противовоспалительные свойства, аналогичные свойствам диклофенака после непрерывного введения.