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Research Article

Experimental Evaluation of the Analgesic Activity of 2-(3-Diethylcarbamoyl-2-Methyl-5-Phenyl-Pyrrol-1-Yl)-3-Phenyl-Propionic Acid

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ABSTRACT

Background: Non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of inflammation and acute and chronic non-malignant pain. A large number of classic and contemporary NSAIDs contain a pyrrolic ring in their structure. In our study, 2-(3-Diethylcarbamoyl-2-methyl-5-phenyl-pyrrol-1-yl)-3-phenyl-propionic acid (compound 2f), an N-pyrrolylcarboxylic acid derivative structurally related to Celecoxib, is evaluated for antinociceptive properties after single and multiple (14 days) administration using animal pain models. Materials and methods: 40 Wistar rats are divided in 5 groups (n=8), treated with saline (control group), Metamizole 200 mg/kg b.w., compound 2f 10, 20 and 40 mg/kg b.w. intraperitoneally. The nociceptive tests employ thermal (plantar and tail flick test) and chemical (formalin test) stimuli. Criteria for analgesic effect are increased latency in plantar and tail flick test and decreased paw licking time in formalin test. Results: Compound 2f increased nociceptive response latency in plantar test and decreased hind paw licking during first and second phase of the formalin test compared to the animals, treated with saline. Compound 2f did not increase reaction time in tail flick test. Conclusions: In conclusion, compound 2f has analgesic properties and they are dose and time dependent. The induced analgesia has increased duration after continuous administration and is most probably mediated by supraspinal and peripheral mechanisms.

Keywords: N-pyrrolylcarboxylic acid; NSAIDs; coxibs; analgesia; animal pain models

INTRODUCTION

Pain changes the quality of life of patients and leads to loss of daily vital functions as well as reduced physical activity, reduced working capacity, insomnia[1]. Drugs of choice in cases of acute and chronic non-malignant pain are non-steroidal anti-inflammatory drugs (NSAIDs)[2]. NSAIDs are a heterogenic group of compounds that share certain therapeutic effects and adverse drug reactions[3]. Non-steroidal anti-inflammatory drugs are one of the most often prescribed drug preparations[4]. In the global pharmaceutical market, they occupy fourth place after antibiotics, cardiovascular and psychotropic medications. In 2010 the market for non-prescription NSAIDs in Central Europe reached 10.7 billion euros, an increase of approximately 13% compared to the previous one-year period[5]. Even though NSAIDS are extremely beneficial for reducing inflammation and pain, their use is often accompanied by unwanted side effects such as: Gastrointestinal – dyspepsia, nausea, abdominal pain, ulcerations. These adverse GI effects can be present in up to 40% of NSAIDs users[6]. Ulcers can be single or multiple, uncomplicated or complicated by bleeding and perforation. The blood loss may be gradual, which over time leads to anemia, or acute and life-threatening[4,6]. Cardiovascular. To improve the safety profile with respect to the gastrointestinal tract cyclooxygenase-2 (COX-2) selective NSAIDs were developed. Placebo-controlled trials, however, have demonstrated an increased risk of myocardial infarction, stroke, thrombosis in this group[7-9]. Analgesic nephropathy[3,10]. Hypersensitivity. Some individuals show hypersensitivity to aspirin and NSAIDs, manifested as vasomotor rhinitis, generalized urticaria, bronchial asthma and laryngeal edema, bronchospasm, hypotension and shock[3,11]. Hepatotoxicity[3,12]. Despite the presence of gastroprotective drugs, mortality associated with the use of NSAID-induced ulcers remains high during the last decade[6]. The entry into clinical practice of selective COX-2 inhibitors (coxibs) gave hope for reducing gastro-intestinal toxicity compared to non-selective NSAIDs. Their continued use, however, was found to cause an increased cardiovascular risk, and several of them were withdrawn from the market[7-9]. This necessitates a search for new drug preparations with analgesic and anti-inflammatory effects. A subject of research in a number of scientific developments has become the pyrrole heterocycle due to its possession of varied biological

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activity at a relatively low toxicity[13,14]. Pyrrolic compounds exhibit different pharmacological effects: antifungal[15], antitumor[16], antimicrobial[17,18], antioxidant[19]. Pyrrolic structures are a viable baseline start in the design of compounds with underlined anti-inflammatory and analgesic effect[20]. Tolmetin and Zomepirac - a hit in anti-inflammatory therapy in the 1980s and 1990s, as well as the drug Klopirac were created and approved after the works of Carson[21,22]. The common characteristic of these medications is the presence of a pyrrolic cycle[23]. The presence of the same heterocycle and its importance for anti-inflammatory and analgesic activity[20,24] is confirmed in a number of current studies[25-28]. 2-(3-Diethylcarbamoyl-2-methyl-5-phenyl-pyrrol-1-yl)-3-phenyl-propionic acid (compound 2f) is a newly synthesized N-pyrrolylcarboxylic acid derivative. (Fig. 1.). The chemistry, design, synthesis and characterization by spectroscopy and TLC (thin line chromatography) are described by Vladimirova et al in "An access to new *N*-pyrrolylcarboxylic acids as potential COX-2 inhibitors via Paal-Knorr cyclization"[29]. 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[30-34]. The structure of the target molecule is designed to include common elements of the pharmacophore systems of current types of NSAIDs, expecting manifestation of their characteristic pharmacological activity. In this way the structural combination is oriented to the architecture of the selected prototype for a contemporary anti-inflammatory agent Celecoxib (CAS 169590-42-5, a selective COX-2 inhibitor). The aim of this study is to evaluate the antinociceptive properties of 2-(3-Diethylcarbamoyl-2-methyl-5-phenyl-pyrrol-1-yl)-3-phenyl-propionic acid (compound 2f) after single and multiple (14 days) administration using animal pain models.

MATERIALS AND METHODS

The experiment is approved by the Ethics Committee on Animals of the Bulgarian Food Safety Agency with permit № 128/09.12.2015 and by decision of the Ethics Committee at the Medical University of Plovdiv, protocol № 2/31.03.2016.

Agents. Metamizole sodium amp. 500mg/ml 2ml (Sopharma AD, Bulgaria); NaCl 0.9% (Sopharma AD, Bulgaria); Formalin 0,2%; 2-(3-Diethylcarbamoyl-2-methyl-5-phenyl-pyrrol-1-yl)-3-phenyl-propionic acid (compound 2f). All substances are dissolved in saline. The drugs are administered intraperitoneally (i.p.) with the doses expressed as milligram salt per kilogram body weight (mg/kg). Experimental animals. 40 adult male Wistar rats weighing 150±20 grams are used, randomly divided in 5 parallel experimental groups with a number of 8 animals per group as follows:

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

group – positive control, treated with referent analgesic Metamizole 200 mg/kg b.w. (i.p.);

group – treated with compound 2f 10 mg/kg b.w. (i.p.);

group – treated with compound 2f 20 mg/kg b.w. (i.p.);

group – treated with compound 2f 40 mg/kg b.w. (i.p.).

The animals are maintained on a light-dark cycle of 12:12 h in a temperature-controlled environment with food and water available ad libitum. The doses used in the experiment are determined according to acute oral toxicity tests. Plantar test (Hargreaves method). The test animal is placed in an individual compartment and is left unrestrained. After an acclimation period an infrared heat source (Ugo Basile, Italy) is positioned under the glass floor directly beneath the hind paw of the animal and paw withdrawal latency (in seconds) is recorded automatically. The chosen infrared intensity is 50 mW/cm^2 and the cut-off time is set at 30 seconds so as to avoid unnecessary overheating of the paw. A specific filter cuts off the visible part of the light spectrum, which could disturb the animal or provide an unwanted clue. Tail flick test. The rat is held by the operator so that the radiant heat source (Ugo Basile, Italy) is focused on the underside of the tail approx. 3 cm from its distal end. The time in seconds for tail withdrawal is automatically recorded. The chosen infrared intensity is 80 mW/cm^2 and the cut-off time is set at 15 seconds to avoid tissue damage. The tests are performed at the first, second and third hour after administration of the test substances. Criterion for analgesic action is prolongation of the reaction time compared to the animals, treated with saline. Formalin test. 0,2% 200µl formalin is injected intradermally in one of the hind paws one hour after the administration of the tested substances. The time for licking the injected paw (in seconds) is counted during the first 10 minutes and at the 20-30th minute after injection. Criterion for analgesic effect is decreased paw licking time in comparison to the control group. Statistical evaluation of the results is performed with IBM SPSS 20.0 software. The normality of distribution is determined with the Kolmogorov-Smirnov test. For each indicator are determined arithmetic mean and standard error of the mean (± SEM). Comparison of the results between groups is done using the Independent Sample T test. A P value < 0,05 is considered statistically significant.

RESULTS

The reference analgesic Metamizole showed significant analgesic effect in all tests after both single and multiple administration. In the plantar test after single administration compound 2f in dose 10 mg/kg b.w. significantly increased paw withdrawal time at first hour, compared to the control group. Dose 20 mg/kg b.w. prolonged the reaction time at first and second hour in comparison to the control animals. Compound 2f 40 mg/kg b.w. increased withdrawal latency at first, second and third hour, compared to the animals treated with saline. After multiple (14 days) administration in the plantar test substance 2f 10 mg/kg b.w. significantly prolonged the time for paw withdrawal at first hour, second and third hour compared to the control animals. Compound 2f in dose 20 mg/kg b.w. increased reaction time at first, second and third hour, compared to the animals, treated with saline. Dose 40 mg/kg b.w. increased the nociceptive response latency at first, second and third hour, in comparison to the control group. (Tab. 1.) A significant

Table 1: Comparison of the results between the control group and the groups, treated with Metamizole and compound 2f in doses 10, 20 and 40 mg/kg b.w. in plantar test.

Group	Number of animals	Hour	x±Sx	t	P	$x_1 \pm Sx_1$	t_1	p_1
Control	8	1st	6,75±0,48	-	-	7,13±1,42	-	-
		2nd	8,67±2,34			7,70±1,72		
		3rd	10,00±2,10			7,17±2,22		
Metamizole	8	1st	17,11±1,97	5,12	0,002*	19,36±2,06	4,55	0,001*
		2nd	17,10±1,22	3,34	0,007*	16,30±2,66	2,50	0,028*
		3rd	18,19±2,24	2,63	0,023*	13,28±1,37	2,47	0,030*
2f 10 mg/kg	8	1st	14,98±0,61	10,03	<0,001*	15,90±0,67	5,59	<0,001*
		2nd	12,34±0,80	1,67	0,12	13,77±0,16	3,51	0,017*
		3rd	12,51±1,07	1,15	0,27	14,65±1,28	2,92	0,015*
2f 20 mg/kg	8	1st	15,70±0,81	9,05	<0,001*	17,22±2,24	3,81	0,003*
		2nd	14,43±1,35	2,22	0,049*	13,93±1,48	2,75	0,021*
		3rd	14,47±1,00	2,02	0,07	15,52±2,04	2,77	0,02*
2f 40 mg/kg	8	1st	17,77±1,10	8,64	<0,001*	19,89±2,83	4,03	0,002*
		2nd	18,59±2,49	2,87	0,015*	13,99±1,04	3,29	0,006*
		3rd	16,06±1,77	2,23	0,048*	16,93±1,75	3,51	0,004*

x±Sx, t, p – values after single administration; $x_1 \pm Sx_1$, t_1, p_1 – values after multiple (14 days) administration; $p < 0,05$*

Table 2: Comparison of the results between the control group and the groups, treated with Metamizole and compound 2f in doses 10, 20 and 40 mg/kg b.w. in formalin test.

Group	Number of animals	Phase	x±Sx	t	P	$x_1 \pm Sx_1$	t_1	p_1
Control	8	1st	70,17±16,81	-	-	42,00±9,36	-	-
		2nd	47,83±11,12			35,00±5,22		
Metamizole	8	1st	23,83±4,35	2,67	0,039*	7,38±3,05	3,96	0,002*
		2nd	18,83±4,07	2,45	0,048*	11,25±3,90	3,73	0,003*
2f 10 mg/kg	8	1st	16,50±2,62	3,15	0,024*	9,00±2,14	3,44	0,016*
		2nd	16,83±3,44	2,66	0,038*	18,00±3,41	2,81	0,017*
2f 20 mg/kg	8	1st	15,43±3,96	3,17	0,021*	8,33±1,69	3,54	0,005*
		2nd	16,71±2,90	2,71	0,037*	16,67±4,43	2,68	0,023*
2f 40 mg/kg	8	1st	10,14±1,37	3,56	0,016*	3,50±0,92	4,09	0,009*
		2nd	6,71±2,40	3,62	0,013*	8,33±3,16	4,37	0,001*

*x±Sx, t, p – values after single administration; $x_1 \pm Sx_1$, t_1, p_1 – values after multiple (14 days) administration; *p < 0,05*

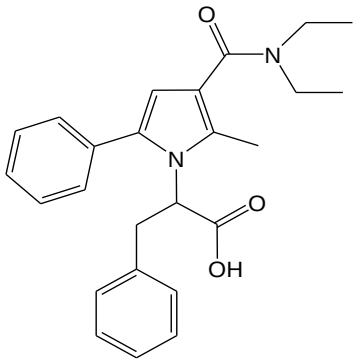

Figure 1: Structure of compound 2f.
2-(3-Diethylcarbamoyl-2-methyl-5-phenyl-pyrrol-1-yl)-3-phenyl-propionic acid

difference in results in plantar test between compound 2f and Metamizole occurred only at dose 10 mg/kg b.w. at second (t=3,34, p=0,05) and third (t=2,38, p=0,033) hour after single administration, where reaction time in the animals treated with compound 2f 10 mg/kg b.w. was lower. All other tested doses after both single and continuous administration showed results similar to those of Metamizole. Compound 2f 10 mg/kg b.w. significantly decreased first and second phase hind paw licking time in the formalin test after single administration, compared to the control group. Compound 2f 20 mg/kg b.w. reduced paw licking time in the first and second phase of the test in comparison to the animals, treated with saline. Dose 40 mg/kg b.w. decreased first and second phase hind paw licking in the formalin test, compared to the control animals. After continuous (14 days) administration compound 2f 10 mg/kg b.w. considerably reduced first and second phase hind paw licking time in the formalin test, in comparison to the rats, treated with saline. Compound 2f 20 mg/kg b.w. decreased paw licking time in the first and second phase of the test compared to the animals of the control group. Dose 40 mg/kg b.w. decreased first and second phase hind paw licking in the formalin test, compared to the control animals. (Tab. 2.). Compound 2f 40 mg/kg b.w. significantly reduced paw licking time compared to the group, treated with Metamizole, in first (t=3,21, p=0,08) and second (t=2,66, p=0,022) phase of the

Table 3: Comparison of the results between the control group and the groups, treated with Metamizole and compound 2f in doses 10, 20 and 40 mg/kg b.w. in tail flick test.

Group	Number of animals	Hour	x±Sx	t	P	x_1±Sx_1	t_1	p_1
		1st	3,33±0,22			3,50±0,70		
Control	8	2nd	2,58±0,10	-	-	2,70±0,25	-	-
		3rd	3,17±0,45			3,93±0,48		
		1st	5,14±0,70	2,47	0,042*	5,80±0,67	2,37	0,039*
Metamizole	8	2nd	3,79±0,39	2,77	0,018*	4,42±0,65	2,47	0,033*
		3rd	4,19±0,79	1,08	0,31	4,32±0,97	0,36	0,73
		1st	3,05±0,35	0,63	0,54	2,28±0,25	1,63	0,13
2f 10 mg/kg	8	2nd	2,48±0,22	0,39	0,70	2,53±0,27	0,45	0,66
		3rd	2,64±0,20	1,18	0,26	2,62±0,41	2,09	0,06
		1st	3,16±0,57	0,27	0,79	2,85±0,52	0,75	0,47
2f 20 mg/kg	8	2nd	2,70±0,22	0,46	0,66	2,75±0,22	0,15	0,88
		3rd	3,19±0,39	0,03	0,98	2,70±0,33	2,14	0,058
		1st	4,08±0,31	1,97	0,08	3,10±0,23	0,54	0,61
2f 40 mg/kg	8	2nd	3,10±0,26	1,88	0,11	2,95±0,39	0,49	0,63
		3rd	3,97±0,59	1,08	0,31	3,16±0,22	1,60	0,14

*x±Sx, t, p – values after single administration; x_1±Sx_1, t_1, p_1 – values after multiple (14 days) administration; * p < 0,05*

formalin test after single administration. In the tail flick test substance 2f in all tested doses after both single and multiple (14 days) administration showed no significant increase in tail withdrawal latency. (Tab. 3.). The results of the control group in this test were comparable to those of compound 2f, therefore a significant difference between Metamizole and compound 2f was also observed. Reaction time in the group, treated with compound 2f 10 mg/kg b.w. was significantly lower compared to Metamizole at first and second hour, both after single (t=2,78, p=0,016; t=3,01, p=0,01) and continuous (t=4,89, p=0,01; t=2,67, p=0,023) administration. Dose 20 mg/kg b.w. also showed significantly lower results in comparison to Metamizole at first and second hour after single (t=2,21, p=0,048; t=2,43, p=0,032) and multiple (t=3,47, p=0,06; t=2,43, p=0,036) administration. Compound 2f 40 mg/kg b.w. demonstrated shorter latency time compared to Metamizole at first hour (t=4,25, p=0,01) after continuous administration.

DISCUSSION

The results in our study show that in experimental conditions 2-(3-Diethylcarbamoyl-2-methyl-5-phenyl-pyrrol-1-yl)-3-phenyl-propionic acid has analgesic action against thermal and chemical stimuli. This effect is registered after both single and multiple administration of the compound. The Hargreaves test is developed to deliver a more localized heat on unrestrained animals and offers the advantage of minimizing the role of subjective factors[35]. Compared to the widely used hot plate method which is very susceptible to learning phenomena that result in a progressive shortening of the reaction time, the plantar test provides no such unwanted clues[36]. In this test compound 2f in all used doses showed significant analgesic effect that is both time and dose dependent. The behavioral reflex response in this test is mediated by supraspinal structures[36], hence we can assume that supraspinal pathways are involved in the analgesia induced by compound 2f. After continuous administration the observed analgesic effect in the plantar test does not weaken and is with increased duration which could indicate accumulation of the compound and lack of tolerance development. Intraplantar administration of formalin leads to biphasic pain response. The initial phase occurs during the first ten minutes and is a result of direct activation of peripheral nociceptors. The second phase begins after a ten-minute quiescent period and is attributed to the release of local endogenous mediators, leading to peripheral inflammatory processes and subsequent sensitization of nociceptive spinal neurons[37]. In our study all tested doses of compound 2f showed significant antinociceptive effect in the first and second phases of the formalin test. We can speculate that peripheral mechanisms are also implicated in the mode of antinociceptive action of compound 2f. That would be in line with the expected of this substance cyclooxygenase inhibiting activity. The analgesia shown by the compound in the second phase of the formalin test could result from either suppression of pro-inflammatory mediators or a modulation of pain transmission on spinal level. That is why we chose the tail flick test to clear possible mechanisms of action further. The tail-flick mediates a spinal reflex to nociceptive stimuli[35]. The measurements are considered precise since the test is measured electronically, which is necessary since the intensity of the stimulus results in very short latencies[38]. For tail-flick the locus of tail stimulation is important for the effect, with the distal section being the more sensitive area[39]. The advantages of this method are its simplicity and the small interanimal variability in reaction time measurements[36]. Compound 2f showed no analgesic activity in this test. Therefore, we can speculate that the antinociception showed in the second phase of the formalin test is only due to inhibition of inflammation and not due to involvement of spinal reflexes. Further investigations with *in vitro* studies of COX-1/COX-2 activity and selectivity of the compound and possible involvement of opioidergic mechanisms are necessary to establish the specific mechanisms of antinociceptive action of compound 2f.

Further study of possible biological activities of the compound is also necessary.

CONCLUSIONS

Compound 2f induces antinociceptive effect after single and multiple (14 days) administration in rats. This effect is dose dependent and time dependent. After multiple (14 days) administration the analgesic effect of compound 2f is long-lasting.

Antinociception in the tested compound is most likely mediated not only by supraspinal pathways, but also by peripheral and anti-inflammatory mechanisms. Regulation of pain transmission in compound 2f does not occur on spinal level.

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