Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ANTINOCICEPTIVE ACTIVITY OF A NEW NON-OPIOID ANALGESIC PYRODAZOL AND KETOROLAC

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ABSTRACT

The search for new analgesics that exceed existing analogues in efficacy and/or safety is important. Existing drugs do not fully meet the modern clinic requirements because of side effects and lack of effectiveness. In the search for new analgesics, antinociceptive activity of the new derivative of 5H-pyrrollo[1,2-a]imidazole (pyrodazol) was examined in comparison with that of ketorolac. Anti-inflammatory (antiexudative) activity was evaluated in the model of carrageenan edema. Analgesia was evaluated in the models of carrageenin edema, Randall and Selitto and acetic acid-induced writhing on nonlinear white mice and Wistar rats after a single orally administration. Pyrodazol is not shown an antiexudative action in the model of carrageenan edema at doses of 1, 5 and 10 mg/kg. In the model of acetic acid-induced writhing, pyrodazol at a dose of 2.5 mg/kg showed an antinociceptive effect up to 2 hours, and in the peak of analgesia (60 min) in a dose range of 0.5 - 5 mg/kg it was superior to ketorolac: ED_{50} 0.92 (0.48 - 1.75) mg/kg and 2.9 (1.45 - 5.8) mg/kg, respectively. In the model of Randall and Salitto in the dose range of 1-20 mg/kg, pyrodazol showed a reliable antinociceptive effect lasting up to 3 hours at dose of 5 mg/kg significantly outperforming the similar effect of ketorolac. The data obtained suggest that in the model of peripheral pain, pyrodazol outperforms ketorolac. An antinociceptive activity of pyrodazol isn`t associated with an effect on COX isoenzymes.

KEYWORDS: Pyrodazol, ketorolac, analgesia, derivative of pyrrollo-imidazole.

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INTRODUCTION

Pain is often associated with inflammation that arises as a result of tissue damage. Tissue damage causes a release of inflammatory mediators (prostaglandins, bradykinin, histamine, etc.), which leads to activation and sensitization of the receptors, and also affects the neurons conducting nociceptive stimuli[1,2,3]. These processes may lead to central sensitization and hypersensitivity. According to modern ideas, pain accompanied by an inflammatory component is a complex pathological condition. Existing treatments for this disease have limited efficacy and/or poor tolerability, especially in chronic diseases such as rheumatoid arthritis and osteoarthritis[4]. Agonists of μ-opioid receptors (MOR) are critical for the treatment of moderate or severe pain. Although MOR agonists are very effective in acute pain, their effectiveness is limited under chronic pain conditions, including chronic inflammatory pain, due to its ability to cause physical and psychological dependence, as well as drug abuse[5,6]. Another group of medications for the treatment of pain, particularly inflammatory pain, includes non-steroidal anti-inflammatory drugs, inhibitors cyclooxygenase-2 (COX-2). However, both classes of drugs do not have sufficient analgesic efficacy [4]. Furthermore, they have inherent side effects on the gastrointestinal tract, nephrotoxicity, hematotoxicity, the risk of side effects of the cardiovascular system, etc.[7]. Thus, there exists a need for drugs with a higher efficiency and improved tolerability profile being effective for chronic pain, including pain with an inflammatory component[8].

Pyrodazol (1,3-di(4[1]-methoxyphenyl)-6,7-dihydro-5H-pyrrollo[1,2-a]imidazol) is a new centrally acting analgesic. To further examine the performance of pyrodazol analgesia profile we studied its activity in models of peripheral pain with an inflammatory componen, in comparison with known and widely used ketorolac.

MATERIALS AND METHODS

Animals

The studies were conducted on male nonlinear mice (20–35 g) and Wistar rats (150–200 g). The animals were housed under quarantine for 7 days before the experiment began. The animals had free access to a standard commercial diet and water. The animals were kept under a stable regimen of 12 hrs light/12 hrs darkness. All studies were performed in accordance with the requirements of the State Expert Center of the Ministry of Health of Ukraine and the rules of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purpose." (Strasbourg, 1986)

Substances

Ketorolac tromethamine (ketorolac) substance (JSC "Lek-Chem", Ukraine), pyrodazol substance (synthesized in the Department of Synthesis of biologically active substances at SI "Institute of pharmacology and toxicology of NAMS of Ukraine", Kiev), carrageenan (Sigma-Aldrich, USA), acetic acid ("Khimlaborreaktiv", Ukraine). Pyrodazol was administered once orally (p.o) in a form of the aqueous-ethanol emulsion using Twin-80 as an emulgator. Ketorolac was administered once orally (p.o) in a form of aqueous solution.

Carrageenan-induced inflammatory in mice

Pyrodazol was administrated p.o. to mice at doses of 1, 5 or 10 mg/kg 1 hr before subplantar injection of 0.05 ml of 1% carrageenan[9]. The experiment group size was 6 animals. 3 h after carrageenan injection, the mice were withdrawn from the experiment. Hind paws with swollen and not-swollen feet were amputated at the level of hip joints. The control group was treated with a solvent. The control group size was 6 animals. The inhibition of edema was calculated using the formula:

$$\text{Inhibition of edema } \% = (M_{se}-M_{he})/(M_{sc}-M_{hc}) *100\%-100\%$$

Where, M_{se}=mass of a swollen foot in the experiment; M_{he}=mass of a healthy foot in the experiment; M_{sc}= mass of a swollen foot in the control; M_{hc}= mass of a healthy foot in the control.

Carrageenan-induced inflammatory pain in rats

This model of acute inflammatory pain was conducted according to Randall and Selitto[10]. The group size was 6 animals. Acute inflammation was induced by injection of 0.1 ml of carrageenan solution (0.5% in distilled water) subcutaneously into the plantar surface of the right hind paw of the rat. The mechanical nociceptive threshold was measured using an Algesiometer (Ugo Basile, Comerio, Italy). The device generates a mechanical force with a linear increase over time. The force was applied to the dorsal surface of the inflamed hind paw via a cone-shaped stylus with a rounded tip (3 mm^2)[11]. The nociceptive threshold (T_V) was defined as the force at which the rat vocalized (cutoff force 450 g)[11]**.** Compounds or vehicle were given 3 h after carrageenan injection. The mechanical nociceptive threshold was measured 30, 60, 45, 90, 120, 150 and 180 min after drug or vehicle administration (pyrodazol and ketorolac were administered p.o. in doses of 1, 5, 20 mg/kg). The analgesic activity of drug is expressed as percentage of the maximal possible effect based on the following formula:

$$\text{Analgesic activity, \%} = T_{Vexp}/Tv_{bas} \times 100\% - 100\%$$

Where, Tv_{bas} - baseline latency; T_{Vex}- latency after drug administration.

Acetic acid induced writhing model in mice

Analgesic activity of pyrodazol and ketorolac was assessed by reduce the number of writhes induced by intraperitoneal injection of 0.6% acetic acid. The number of writhes per animal was counted for 10 min[12]. Inhibition of writhing was calculated by the formula below and compared with the standard drug (ketorolac)

$$\text{Inhibition of writhing, \%} = \{(Wc - Wt) \times 100\%\}/Wc$$

Where, Wc = number of writhing in the control group; Wt = number of writhing of the treated group.

There were two series of experiment.

In first series for studying of dynamics of pyrodazol analgesic effect of at doses of 2.5 mg/kg, p.o. animals were pretreated with drugs 30, 60, 90 and 120 min before induction of writhing. The control group was given a solvent, p.o.. The experiment groups size was 6 animals. The control group size was 10 animals. In the second series of experiment for evaluation of analgesic activity of pyrodazol at the doses of 0.5; 0.7; 1 and 5 mg/kg, p.o. in comparison with ketorolac 1; 2.5 and 5 mg/kg, p.o. animals were pretreated with drugs 60 min (maximum of analgesia) before induction of writhing. The control group was given a solvent, p.o. The experiment groups size was 6 animals. The control group size was 10 animals.

Statistics

ED_{50} of pyrodazol and ketorolac in the studied models was calculated using the Litchfield-Wilcoxon method[13]. The statistical analysis of the data obtained was performed by the method of variation 0 statistics (t-test) OriginPro 8.0 (originLab Corporation, USA)[14].

RESULTS

Pyrodazol did not show an antiexudative effect in the carrageenan edema model on mice at the dose range 1 to 10 mg/kg (Table 1).

Table 1
Antiexudative effect of pyrodazol at the model of carrageenan edema on mice

Group	ΔM, mg	Inhibition of edema, %
Pyrodazol, 1 mg/kg	6.30±0.20	-1.56
Pyrodazol, 5 mg/kg	5.30±0.51	-17.18
Pyrodazol, 10 mg/kg	6.10±0.78	-4.68
Control	6.40±0.78	**-**

In applying pyrodazol in the acetic acid-induced writhing test on the mice at the dose of 2.5 mg/kg, a peak of an analgesic effect was determined 60 min after administration. Up to 90 min, analgesia decreased, and it was absent 120 min after pyrodazol administration (Table 2). An antinociceptive reaction was registrated between 60 and 90 min after pyrodazol administration. In the second group of experiments, a comparative study of the pyrodazol efficacy (dose range 1 to 5 mg/kg) was conducted at an indicative peak of analgesia. For this purpose, pyrodazol and ketorolac as reference drug were administered 60 min before injection of acetic acid and a number of writhing in the control and treated groups was recorded. It is found that pyrodazol, depending on the dose, inhibits number of writhing from 32.3 % to 55.9% (Table 3).

Table 2
Dynamics of the analgesic effect of pyrodazol in the model of acetic acid-induced writhing on mice

Group	Statistic	Number of writhing			
		30 min	60 min	90 min	120 min
Control,n=10	M±m	19.8±0.47	19.8±0.47	19.8±0.47	19.8±0.47
Pyrodazol, 2.5 mg/kg , n=6	M±m	22.8±0.67	11.8±0.30	12.4±0.32	19.8±0.40
	Changes compared to the control, %	+15.2	-40.4*	-37.3*	0

Note. * - Significant changes compared to the control (P<0.05)

Table 3
Comparative evaluation of analgesic activity of pyrodazol and ketorolac in the model of acetic acid-induced writhing on mice 60 min after drugs administration

Group	Number of writhing, M±m		Inhibition of writhing,%
	Control group, n=10	Experimental group, n=6	
Pyrodazol, 0.5 mg/kg	25.4±0.56	17.2±0.19	-32.3*
Pyrodazol, 0.7 mg/kg	21.7±1.98	12.3±1.87	-43.3*
Pyrodazol, 1.0 mg/kg	25.4±0.56	11.2±0.46	-55.9*
Pyrodazol, 5 mg/kg	25.4±0.56	14.2±0.19	-44.1*
Ketorolac, 1 mg/kg	32.6±4.59	17.6±5.74	-46.0*
Ketorolac, 2.5 mg/kg	32.6±4.59	15.8±5.00	-51.5*
Ketorolac, 5 mg/kg	32.6±4.59	14.2±4.19	-56.4*

Note. * - Significant changes compared to the control (P<0.05)

In the Randall and Sellitto test, the ability of pyrodazol and ketorolac to change paw withdrawal latency in response to the increasing dosed pressure was tested. It is found that under a mechanical nociceptive stimulation pyrodazol and ketorolac cause a significant increase in pain threshold as soon as 30 min after p.o. administration (Table 4). Depending on the dose, the antinociceptive effect lasts up to 3 hours. Pyrodazol shows a significant antinociceptive effect: analgesia reaches +327.2% after administration at a dose of 5 mg/kg. It is shown that the “dose-effect” dependence for pyrodazol is nonlinear, unlike that of ketorolac.

Table 4
Analgesic activity of pyrodazol and ketorolac in the test of mechanical nociceptive stimulation (Randall and Selitto test) on rats

Group	Statistic	Baseline value	Time after administration					
			30 min	60 min	90 min	120 min	150 min	180 min
Pyrodazol, 1 mg/kg	M±m	5.60±0.96	9.30±3.95	7.10±1.44	7.50±4.42	5.60±0.91	5.60±1.71	-
	changes compared to the baseline value, %		+66.1*	+26.8	+33.9	0	0	-
Pyrodazol, 5 mg/kg	M±m	4.37±2.80	15.50±4.26	14.30±4.56	6.21±1.38	10.39±3.90	11.72±4.33	8.00±4.20
	changes compared to the baseline value, %		+254.6*	+327.2*	+42.1	+137.7*	+168.1*	+83.1*
Pyrodazol, 20 mg/kg	M±m	6.88±1.39	15.13±5.71	7.50±1.47	14.25±6.21	8.38±5.54	-	-
	changes compared to the baseline value, %		+119.9*	+9.0	+107.1*	+21.8	-	-
Ketorolac, 1 mg/kg	M±m	5.37±1.60	7.60±1.68	6.90±1.53	-	-	-	-
	changes compared to the baseline value, %		+41.5*	+28.5	-	-	-	-
Ketorolac, 5 mg/kg	M±m	11.00±3.90	17.30±4.72	17.60±4.76	20.8±4.20	16.80±5.04	-	16.30±3.99
	changes compared to the baseline value, %		+57.3*	+60.0*	+89.1*	+52.7*	-	+48.2
Ketorolac, 20 mg/kg	M±m	3.75±0.43	15.13±5.70	-	7.50±0.75	5.13±2.09	-	-
	changes compared to the baseline value, %		+303.5*	-	+100.0*	+36.8		

Note. M±m – the average latency, (n = 6)
**** Significant changes compared with the baseline value, P<0.05 (a nonparametric "sign test" wasused for evaluation)***

DISCUSSION

Analgesics that are used today, whose mechanism of action is associated with the effect on COX isoenzymes, have significant side effects[15]. Therefore, it is important to search new analgesics exceeding the existing ones in efficacy and/or safety. This study was conducted to examine the possible peripheral action of new analgesic – pyrodazol. The study is a fragment of the determination of the efficiency of pyrodazol both in acute and chronic pains. Pain syndrome that occurs in intraperitoneal injection of acetic acid in mice is manifested through a peculiar abdominal constriction (writhing). It is associated with irritation of the peritoneum and is regarded as a model of visceral pain. In this case, intraperitoneal injection of 0.6% acetic acid solution assists a general activation of nociceptive system and local release of bradykinin, histamine, serotonin, prostaglandins, etc.[16]. Acetic acid causes an increase in peritoneal fluid levels of prostaglandins (PGE2 and PGF2α), involving in part peritoneal receptors [17] and inflammatory pain by inducing capillary permeability[18]. Calculated values of ED_{50} of pyrodazol and ketorolac at the analgesia peak (60 min after p.o. administration), were respectively 0.92 (0.48 - 1.75) mg/kg and 2.9 (1.45 - 5.8) mg/kg. The above result indicates that in the model of nociceptive stimulation (writhing caused by intraperitoneal injection of acetic acid) pyrodazol out performs ketorolac by about 3 times. Carrageenan edema characterizes the cyclooxygenase path way of inflammation[19]. However, in the model of carrageenan edema, an anti-exudative effect of pyrodazol in the studied doses was not detected. But in the mechanical nociceptive stimulation model of Randall and Selitto pyrodazol shows a reliable antinociceptive effect. Analgesic effect was dose-dependent. Maximum effect and its duration were registered after administration of pyrodazol at a dose of 5 mg/kg (Table 3). In this case effectiveness of 5 mg/kg pyrodazol exceeded ketorolac in similar dose respectively in 4.4; 5.5; 2.6 and 1.7 times 30, 60, 120, 180 min after administration. Thus obtained data suggest that the antinociceptive effect of pyrodazol is not associated with an effect on COX isoenzymes, at least at the peripheral level. These data do not exclude realization of an antinociceptive effect through other mechanisms (catecholaminergic, etc.)[20].

CONCLUSION

In acetic acid-induced writhing and Randall and Selitto models pyrodazol is superior to ketorolac. ED50 of pyrodazol is 0.92 (0.48 - 1.75) mg/kg against 2.9 (1.45 - 5.8) mg/kg for ketorolac on the acetic acid induced writhing model. Antinociceptive effect of pyrodazol is not associated with an effect on COX.

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