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ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES OF SS-68 INDOLE DERIVATIVE

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Abstract

The experiments with rats show that when administered intravenously (5 mg/kg) and intragastrically (5, 10, 15 and 20 mg/kg), SS-68 compound exerts an anti-inflammatory effect (AIE) on models of acute inflammation, caused by carrageenin and serotonin, and exerts no AIE in case of edema induced by complete Freund's adjuvant (CFA) and histamine, except for intragastric administration at a dose of 20 mg/kg.

By its antiphlogogenic effect in case of carrageenin and serotonin edemas, SS-68 is comparable to diclofenac (5, 10 and 15 mg/kg intragastrically) and indomethacin (10 mg kg intragastriccally), and exceeds piroxicam (20 mg/kg intragastrically). By reference to its therapeutic index (TI), in the first case SS-68 exceeds diclofenac, indomethacin and piroxicam 2.2, 15.6 and 5.6 times, and in the second case it exceeds them 1.8, 12.8 and 4.4 times, respectively.

In the mouse acetic writhing test, which reflects the predominant effect on κ (kappa)-opioid receptors, SS-68 when administered intraperitoneally at doses of 0.03, 0.06 and 0.12 mg/kg causes a dose-dependent decrease in the number of writhes by 40.0, 69.0 and 80.3%, respectively. Butorphanol, used for comparison in doses of 0.03, 0.12, 0.24, 0.48 and 0.96 mg/kg, exerts a dose-dependent analgesic effect (AE), with the number of writhes being 41.5, 52.4, 65.3, 71.1 and 80.6%, respectively. By its analgesic activity and TI, SS-68 exceeds butorphanol 1.9 and 2.7 times, respectively.

Based on the results of the docking of affinity indicators for κ_1-opioid receptors of SS-68, butorphanol (agonist-antagonist), as well as U-50488 compounds (selective agonist), it was established that, in accordance with the calculated values of the binding constant, the affinity in relation to SS-68 was 4.53 times higher than for butorphanol, and when compared with U-50488 it was 2.84 times lower. The suggestion is that AE of SS-68, like that of butorphanol, can be linked with both affinity for κ_1-receptors and for other types of opioid receptors (mu, delta) and κ-receptor subtypes (κ_2 or κ_3).

Keywords: SS-68indole derivative, diclofenac sodium, indomethacin, piroxicam, anti-inflammatory effect, carrageenin, serotonin, histamine, complete Freund's adjuvant, analgesic effect, kappa opioid receptors

Introduction

Earlier, we have shown that SS-68 indole derivative has a high antiarrhythmic (in case of heart rhythm disorders of peripheral and central genesis) and anti-ischemic (antianginal) effect [1]. Besides, it is known that indole derivatives, in particular indomethacin and etodolac [2], have pronounced anti-inflammatory and analgesic properties.

According to [3], of all opioid receptor subtypes, the κ(kappa)-opioid receptor system takes the most significant part in developing the aforementioned pharmacological effects of many substances, and κ-receptor agonists can potentially become promising in terms of being used as a base for creating new medications with antiarrhythmic, antianginal, anti-inflammatory, analgesic and other effects.

In view of the foregoing, it was of interest to study the anti-inflammatory and analgesic (realized through the impact on κ-opioid receptors) effect of SS-68.

The Goal was to evaluate the anti-inflammatory and analgesic effect of SS-68.

Materials and Methods

The SS-68 compound was synthesized at the Department of Natural and High Molecular Compounds at the Southern Federal University (Rostov-on-Don, Russia).

The experiments were carried out on 87 white non-linear male mice and 302 male rats weighing 22-24 g and 210-230 g, respectively. The animals were kept in the standard conditions in a vivarium in accordance with GOST R 50258-92.

Studies of anti-inflammatory and analgesic effects were carried out in compliance with the "The Guidelines for Conducting Pre-clinical Studies of Medications" [4].

To study the effect of SS-68 on exudative inflammation, there were used the models of inflammation caused by carrageenin [5], serotonin, histamine and complete Freund's adjuvant [4]. Phlogogenic substances (made by "Sigma") – carrageenin (1%), serotonin (0.1%), histamine hydrochloride (2 mM) and CFA (0.1 ml) were injected subplantarly into the right hind paw of the rats. The compound SS-68, water-based for injection, was injected intravenously (iv) into the vein of the tail and intragastrically (IG) once for histamine and serotonin-induced edemas and twice in case of edemas caused by carrageenin and CFA. Diclofenac sodium, indomethacin and piroxicam (tablets crushed to homogeneous mass in Tween-80), used as reference preparations, were injected ig once through the probe 1 hour before phlogogens.

The volume of the paws (the right one and the left intact one) was measured oncometrically using a plethysmometer ("UgoBasile", Italy) 4 and 24 hours after carrageenin and CFA administration, 30 and 45 minutes after histamine and serotonin administration, respectively.

For assessing the inflammatory response of the paw, the following formula (1) was used:

$$I = \frac{Vt - Vo}{Vo} \times 100\%, \qquad (1)$$

where I – is an increase in paw volume (%); V_t – paw volume after injection of phlogogen (ml); and V_o – paw volume before phlogogone administration (ml).

The therapeutic effect of SS-68 and reference preparations was evaluated in comparison with the control; the calculations were made by using the formula (2):

$$100\% - \frac{Vt - Vo}{Vo}\ e : \frac{Vt - Vo}{Vo}\ c \times 100\%, \qquad (2)$$

where e – are experimental animals, c – are control animals.

In addition, the antiphlogogenic affect of substances was assessed by average effective doses (ED_{50}) and a range of therapeutic effect – the therapeutic index ($TI = LD_{50} / ED_{50}$).

The analgesic effect was studied using mouse acetic writhing test. The chosen test is intended to study the acute visceral and deep

somatic pain [6, 7].

The SS-68 compound and butorphanol tartrate (JSC Moscow Pharmaceutical Factory, Russia), taken for comparison, both water-based for injections, were administered to the animals once intraperitoneally (ip) to the right lower third of the abdomen in doses ranging from 0.03 to 0.12 mg/kg and from 0.03 to 0.96 mg kg, respectively, 5 minutes before ip (on the left side) injection of 0.6% acetic acid solution (0.1 ml/10 g of body weight) [6]. The degree of analgesic effect (A) of substances was expressed as a percentage, using the following formula (3):

$$A = \frac{Nc-Ne}{Nc} \times 100\%, \qquad (3)$$

where Nc – is the number of writhes in the control group of animals, Ne – in the experimental group.

In addition, the analgesic effect was evaluated by ED_{50} and TI.

Besides, the κ_1-opioid receptor SS-68, butorphanol, a partial agonist of the κ_1 receptors, and U-50488, a highly selective κ_1 receptor agonist, were docked into a specific binding site. The construction of 10 conformations of each compound was carried out in Marvin Sketch 17.1.23 program [8]. These conformations were optimized in MOPAC2016 [9] and the best one with minimal energy was selected. The docking of a human κ_1-opioid receptor (PDB code 4DJH) into an X-ray diffraction dimer model [10] was performed using AutoDock Vina 1.1.1 [11] (each compound was docked 5 times into each of the two dimer sites), along with calculating the minimum docking energy ΔE. The ensemble docking procedure is described in detail in [12].

The calculation for the docking energy ΔE of the binding constants K was carried out using the formula (4):

$$K = e^{-\Delta E/RT}, \qquad (4)$$

where R – is gas constant equal to 8.314 J/mol; T – temperature, the standard value in docking is 300 ^{o}K.

Statistical processing of the data obtained in graded and alternative forms was carried out by means of computer software developed at the Department of Pharmacology of Kuban State Medical University of the Ministry of Healthcare of Russia.

Results and Discussion

SS-68 compound administered iv to rats at a dose of 5 mg/kg caused a marked inhibition of exudative inflammation triggered by phlogogens carrageenin and serotonin, while inhibition of edema compared with the control group was 71.4 and 77.8 %, respectively (Table 1). No inhibition of edema was observed when administering SS-68 in the indicated dose and via the above channel in cases of inflammatory process caused by CFA and histamine (Table 1).

In cases of administering ig SS-68 and diclofenac in doses of 5, 10 and 15 mg/kg, the inhibition of carrageenin and serotonin edema was 36.2 and 27.5, 59.0 and 52.8, 75.2 and 79.2 for the former substance, and 24.5 and 28.4, 52.4 and 54.3, 67.8 and 72.5% for the latter substance, respectively, i.e., in terms of the antiexudative effects of SS-68 and diclofenac in presence of marked phlogogens, they were close. It was of interest to compare the antiphlogogenic effect of SS-68 under the accepted experimental conditions with that of indomethacin and pyroxicam in doses of 10 and 20 mg/kg, which, according to our data and literature data [13], corresponds to ED_{50}. It was found that with carrageenin and serotonin inflammation, indomethacin and piroxicam caused inhibition of edema by 45.4 and 97.1, 54.8 and 40.6%, respectively (Table 1), that is, the antiphlogogenic effect of these drugs was close to that SS-68. The exception was the antiexudative effect of indomethacin in serotonin edema, which was more significant than that of SS-68, as well as of diclofenac and piroxicam.

Table 1

The impact of SS-68, diclofenac, indomethacin and piroxicam on the development of acute exudative edema caused by carrageenin, CFA, serotonin and histamine in rats (M ± m)

Inflammation initiator	Substance under study	No of animals	Dose, mg/kg	Route	Edema increase, %	Edema inhibition, %
Carrageenin	Control	8			59.7 ± 6.5	
	SS-68	8	5	iv	16.9 ± 3.6***	71.7
		10	5	ig	38.1 ± 3.1**	36.2
		10	10	ig	24.5 ± 4.1***	59.0
		10	15	ig	14.8 ± 3.8***	75.2
	Diclofenac	9	5	ig	45.1 ± 2.1*	24.5
		10	10	ig	28.4 ± 4.1***	52.4
		10	15	ig	19.2 ± 3.5***	67.8
	Indomethacin	8	10	ig	32.6 ± 5.2**	45.4
	Piroxicam	8	20	ig	27.0 ± 8.6***	54.8
Serotonin	Control	8			78.8 ± 3.4	
	SS-68	8	5	iv	17.5 ± 6.0***	77.8
		10	5	ig	57.1 ± 4.7**	27.5
		10	10	ig	37.2 ± 4.3***	52.8
		10	15	ig	16.4 ± 3.8***	79.2
	Diclofenac	10	5	ig	56.4 ± 4.8**	28.4
		9	10	ig	36.0 ± 5.6***	54.3
		10	15	ig	21.7 ± 4.2***	72.5
	Indomethacin	8	10	ig	2.3 ± 0.9***	97.1
	Piroxicam	8	20	ig	46.8 ± 5.8**	40.6
CFA	Control	8			147.0 ± 9.0	
	SS-68	8	5	iv	154.9 ± 8.0	-5.4
		8	10	ig	138.7 ± 7.6	5.6
		10	20	ig	105.0 ± 8.2**	28.6
	Diclofenac	10	10	ig	98.6 ± 6.3***	32.7
	Indomethacin	8	10	ig	67.7 ± 5.0***	53.9
	Piroxicam	8	20	ig	112.6 ± 17.6	23.4
Histamine	Control	8			70.0 ± 9.7	
	SS-68	8	5	iv	54.1 ± 9.2	22.7
		10	10	ig	68.3 ± 7.9	2.4
		10	20	ig	37.4 ± 4.5**	46.6
	Diclofenac	8	10	ig	29.0 ± 3.8***	58.6
	Indomethacin	8	10	ig	40.3 ± 8.6*	42.4
	Piroxicam	8	20	ig	60.5 ± 4.7	13.6

Note: * $p< 0.05$, ** $p< 0.01$,*** $p< 0.001$ compared with control

To assess the antiexudative effect of SS-68 and reference preparations, we compared their ED_{50} (calculated for SS-68 and diclofenac and borrowed from literature sources for piroxicam [13]) and TI. It turned out that by activity (ED_{50}) SS-68, diclofenac and indomethacin are almost comparable. As for piroxicam, SS-68 was 2.4 times more significant by this indicator in relation to it. By their TI in the case of carrageenin and serotonin inflammation, SS-68 exceeded diclofenac, indomethacin and piroxicam 2.2, 15.6 and 5.1 times in the former

case, and 1.8, 12.8 and 4.4 times in the latter case, respectively (Table 2).

Table 2

Acute toxicity, anti-inflammatory effect and TI of SS-68, on carrageenan and serotonin models of exudative inflammation in rats

Substance	LD_{50}[1], mg/kg	Exudative inflammation models			
		carrageenan		serotonin	
		ED_{50}, mg/kg	TI	ED_{50}, mg/kg	TI
SS-68	608.0	8.3	73.3	9.5	64.0
Diclofenac	350.0[2]	10.4	33.7	9.6	36.5
Indomethacin	47.0[3] (26.0 ÷ 85.0)	10.0	4.7	9.4	5.0
Piroxicam	290.0[4] (193.0 ÷ 435.0)	20.0[4] (13.0 ÷ 32.0)	14.5	20	14.5

[1] For mice via ig administration
[2]Neugodnova, O.P. et al. [14].
[3] Tsarichenko, G.V., Gan ina, G.A. [15].
[4]Sigidin, Ya.A. et al. [13].
Note: In brackets is confident limit when p=0.05.

With CFA-and histamine-induced edema, SS-68 (iv 5 mg/kg and/or 10 mg/kg) and piroxicam (ig 20 mg kg) showed no statistically significant anti-inflammatory effect, whereas SS-68 (ig 20 mg/kg), diclofenac (ig 10 mg/kg) and indomethacin (ig 10 mg/kg) inhibited the edema induced by the former phlogogen by 28.6, 32.7 and 53.9%, by the latter – by 46.6, 58.6 and 42.4%, respectively.

Our data on the anti-inflammatory effect of SS-68 in the selected models of edema reflecting various pathogenetic mechanisms of acute exudative inflammation (carrageenan edema simulates the prostaglandin phase of inflammation completed by affecting cyclooxygenase; serotonin and histamine edemas simulate the release of the marked biogenic amines increasing vascular permeability from mast cells; CFA-induced edema coincides with the initial phase of inflammation involving cytokines, in particular IL-1, IL-2, IL-6, IL-8, TNF-α, IFN-γ, as well as leukotrienes accumulated at that stage of arachidonic acid metabolism, which is realized via the 5-lipoxygenase pathway [16, 17]) to a certain extent attest to the "universal" mechanism of anti-inflammatory effect of SS-68.

Thus, SS-68 with iv and ig administration can exert an anti-inflammatory (dose-dependent in the latter administration route) effect on models of acute carrageenin- and serotonin-induced inflammation, and exerts no anti-inflammatory effect with CFA- and histamine-induced edema, except cases of ig administration at a dose of 20 mg/kg.

According to antiphlogogenic activity in carrageenan and serotonin edema, SS-68 is comparable with diclofenac and indomethacin and exceeds piroxicam, and by TI it exceeds all the reference preparations.

In the mouse acetic writhing test, which reflects the predominant effect on κ-receptors, the number of writhes in the control group of animals was 29.4 on average. The SS-68 compound at doses of 0.03, 0.06 and 0.12 mg/kg caused a dose-dependent decrease in the number of writhes by 40.0, 69.0 and 80.3%, respectively (Table 3).

Table 3

Analgesic effect of SS-68 and butorphanol with ip administration in mouse acetic writhing test (M ± m)

Substance	No of animals	Dose, mg/kg	Analgesic effect number of writhes	Analgesic effect in relation to control, %
Control	12		29.4 ± 2.8	-
SS-68	10	0.03	17.6 ± 2.6*	40.0
	10	0.06	9.1 ± 1.2**	69.0
	10	0.12	5.8 ± 1.1**	80.3
Butorphanol	10	0.03	17.2 ± 2.4*	41.5
	8	0.12	14.0 ± 1.3**	52.4
	9	0.24	10.2 ± 1.5**	65.3
	8	0.48	8.5 ± 1.6**	71.1
	10	0.96	5.7 ± 1.2**	80.6

Note: * $p < 0,01$, ** $p < 0,001$compared with control

Butorphanol, used for comparison under the experimental conditions, at doses of 0.03, 0.12, 0.24, 0.48 and 0.96 mg/kg had a dose-dependent analgesic effect, with the number of writhes in comparison with that in the control group being 41.5, 52.4, 65.3, 71.1 and 80.6%, respectively (Table 3). The calculation of ED_{50} and TI of SS-68 and butorphanol showed that for the former their values are 0.038 mg/kg and 7276.3, and for the latter – 0.072 mg/kg and 2666.7, that is, by its analgesic effect and TI, SS-68 exceeds butorphanol 1.9 and 2.7 times, respectively (Table 4).

Table 4

Acute toxicity, analgesic effect and TI of SS-68 and butorphanol when administered ip in mouse acetic writhing test

Substance	LD_{50}, mg/kg	ED_{50}, mg/kg	Analgesic effect relative	TI	relative
SS-68	276.5	0.038	1.9	7276.3	2.7
Butorphanol	192.0[1]	0.072	1	2666.7	1

[1]Anisimova, V.A. et al. [18].

To confirm the agonistic effect of SS-68 on κ_1-receptors, we compared the affinity values of SS-68, butorphanol, and U-50488 compound for these receptors, calculated basing on the docking results. It was found that, in accordance with the target value of the binding constant, the affinity of SS-68 for the κ_1-opioid receptors was 4.53 higher than that of butorphanol, and 2.84 times lower than that of U-50488 (Table 5).

Table 5

Results of docking SS-68, butorphanol and U-50488 substance into specific binding site of κ_1-opioid receptor

Substance	Docking energy ΔE, kcal/mol	Binding constant K, nM
SS-68	-8.60	538.1
Butorphanol	-7.70	2437.3
U-50488	-9.20	196.5

It should be noted that U-50488 is more toxic than SS-68: when administered ip to mice, the LD_{50} of the former being 25 mg/kg [19], whereas for the latter – 276.5 mg/kg.

Thus, SS-68 has an analgesic effect, realized through the peripheral level of establishing pain sensitivity through agonistic action on κ_1-receptors. It is possible that the analgesic effect of SS-68, like that of butorphanol, can be linked to affinity for κ_1-receptors, other types of opioid receptors (mu, delta) and for κ-receptor subtypes (κ_2 or κ_3). To identify the possible involvement of certain opioid receptors in the mechanism of analgesic action of SS-68, additional in-depth studies are needed, which will make it possible to classify SS-68 as either selective or non-selective κ-receptor agonist.

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Conflicts of Interest

The authors have no conflict of interest to declare.

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