

EXPERIMENTAL PHARMACOLOGICAL RESEARCHES EVALUATING THE ANALGESIC ACTIVITY FOR NOVEL HYBRID PRODRUGS OBTAINED BY ESTERIFICATION OF NSAIDs COMPOUNDS WITH PROSTAGLANDINIC COMPOUNDS

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Abstract

Safety reevaluation for non-selective cyclooxygenase (COX) inhibitor non-steroidal antiinflammatory drugs (NSAIDs) demonstrated that the risk of inducing gastroduodenal ulcer is extremely high. Literature data show that the long term use of those drugs (piroxicam, naproxen, diclofenac) increases therapy costs by concomitant administration of antiulcer drugs: H₂ antihistamines, proton pump inhibitors, whilst the risk of gastrointestinal hemorrhages is much reduced when non-selective COX inhibitor NSAIDs are administered in association with prostaglandinic derivatives which exhibit gastric cytoprotective effect.

Based on these observations, in the present non-clinical trial we aimed at determining the analgesic efficacy of newly synthesized derivatives noted: S4As, S6As, S8As, obtained by esterification of acetylsalicylic acid with prostaglandinic compounds [synthesized to Romanian National Institute of Chemical-Pharmaceutical Researches, Bucharest]. We have established for these derivatives the maximal tolerated dose of active substance orally administered in mouse without registering adverse reactions and we determined their analgesic efficacy in chemical and thermal stimulus tests.

Rezumat

Reevaluarea siguranței privind utilizarea antiinflamatoarelor nesteroidiene (AINS) inhibitoare neselective ale ciclooxigenazei (COX), a arătat că riscul inducerii ulcerului gastroduodenal este extrem de crescut. Datele din literatură arată că în cazul utilizării pe termen lung a acestor medicamente (piroxicam, naproxen, diclofenac) costul terapiei crește prin utilizarea concomitentă a unor medicamente antiulceroase: antihistaminice H₂, inhibitori ai pompei de protoni. Literatura de specialitate a evidențiat faptul că riscul hemoragiilor gastrointestinale este mult mai redus, atunci când AINS inhibitoare neselective COX se utilizează în asociere cu derivați prostaglandinici, care prezintă efect citoprotector gastric.

De aceea, în acest studiu non-clinic ne-am propus să determinăm eficacitatea analgezică a unor derivați nou sintetizați, notați: S4As, S6As, S8As, obținuți prin esterificarea acidului acetilsalicilic cu compuși prostaglandinici [sintetizați la Institutul

Național de Cercetări Chimico-Farmaceutice, București]. Am stabilit pentru acești compuși doza maximă tolerată de substanță activă administrată oral la șoarece, fără apariția de reacții adverse și am determinat eficacitatea lor analgezică în testul stimulului chimic și în testul stimulului termic.

Keywords: hybrid prodrugs, analgesic

Introduction

Classic non-selective Cyclooxygenase (COX) inhibitor non-steroidal antiinflammatory drugs (NSAIDs) consumption was evaluated in clinical trials [1, 3, 10], registering on one hand their clinical efficacy [1, 5, 8] and on the other hand their toxicity [6,7]. Recent researches [2,4] highlighted the use of NSAIDs in the treatment of colorectal cancer. A meta-analysis of 44 clinical trials, including approximately 400,000 patients treated with NSAIDs, assessed the relative risk of inducing gastric/duodenal cancer at 2.22% for ibuprofen, 3.13% for acetylsalicylic acid and 8.7% for piroxicam [14]. Some of the trials [11] noted a greater incidence of gastric/duodenal cancer in patients treated with diclofenac, piroxicam, naproxen, compared to patients treated with diclofenac-misoprostol (prostaglandinic analogue) association. Considering the chronic large scale use of non-selective COX inhibitor NSAIDs and their risk of induced gastro-intestinal disorders [13], we synthesized new hybrid prodrugs, named S4As, S6As, S8As, by esterification of acetylsalicylic acid with prostaglandinic compounds at the Romanian National Institute of Chemical-Pharmaceutical Researches, Bucharest. For the three newly synthesized compounds, experimental pharmacological researches were conducted for the determination of analgesic activity by two classic tests of analgesia: chemical stimulus test and thermal stimulus test.

Materials and methods

The investigated substances were synthesized at the Romanian National Institute of Chemical-Pharmaceutical Researches, Bucharest and were conventionally nominated S4As, S6As, S8As. Chemically, they are: S4As (the ester of acetylsalicylic acid with D-Cloprostenol 1- isopropyl ester), S6As (the ester of acetylsalicylic acid with 5 β -chloro-3 hydroxy-2[4-(3 chlorphenoxy)-3 oxo-1 trans-butenyl]-cyclopentyl methyl acetate), S8As (the ester of acetylsalicylic acid with 6-(2 hydroxy-cyclopentyl) ethyl hexanoate).

Determination of maximal tolerated dose

The determination of the maximal tolerated dose was performed by administering a single oral dose of 500 mg/kg bw (5%) aqueous suspension, of each investigated substance, in NMR I strain white male mice, in groups of 20 animals. Animals were monitored for 14 days registering the variation of body weight, exterior aspects (fur, mucosities), motor behavior (agitation, sedation), adverse reactions and lethality.

Determination of analgesic effect

Two classic tests were performed: chemical stimulus test (writhing test with 0.6% acetic acid, administered intra-peritoneally i.p.) and thermal stimulus test (hot plate- 53°C) [9, 12, 15]. In both tests, the control group was treated with distilled water (suspension vehicle for the investigated substances), using as reference substance acetylsalicylic acid.

Chemical stimulus test

Acetic acid 0.6% administered intraperitoneally exerts an irritative effect and induces writhings (contortions) in mouse [9, 12, 15]. Drugs with analgesic potential reduce the number of writhings induced by the acid. The animals were divided into 5 groups (10 animals/group) that were orally treated with distilled water 0.1 mL/10g bw for the control group, acetylsalicylic acid 100 mg/kgbw for the reference group and S4As, S6As, S6As (3 investigation groups) 250 mg/kg bw. Forty-five minutes after the substances' administration, acetic acid 0.6% i.p. was administered.

Animals were allowed 3 minutes unmonitored following the acetic acid administration. For each control or treated animal, housed individually in plexiglas cages, writhing movements were counted for 10 minutes.

Writhing was considered as: animal stretching followed by forebody torsion, abdomen retraction and opisthotonus in such manner that hind limbs touch the surface where the animal lies.

Thermal stimulus test

Experimental data from literature showed that time of paw licking in the animals exposed to hot-plate (mice, rats) characterizes the phenomenon of pain perception at thalamic level, serving therefore to determine the analgesic action of the investigated substances [9, 12, 15]. Five groups (10 animals each) were treated orally as follows: distilled water 0.1 mL/10g bw for the control group, acetylsalicylic acid 100 mg/kg bw for the reference group and S4As, S6As, S6As (3 investigation groups) 250 mg/kg-bw. The

licking time was determined initially after the exposure to the hot-plate (53°C) and 45 minutes after the administration of the substances.

Results and discussion

Results were statistically processed by *t* Student test, using Microsoft Excel 2003 and GraphPad Prism 5 software.

None of the treated groups registered lethality at the administered dose, suggesting that toxicity of the investigated compounds following oral administration is low. No alterations in motor or sexual behavior were registered for the treated animals. For neither of the above groups modifications in exterior aspect (fur, mucosities) or adverse effects were noted.

Body weight evaluation for 14 days underlined no statistically significant difference against initial along the determinations, respecting increasing or decreasing trends depending on food consumption similar to untreated animals (table I).

Table I
Body weight dynamics for animals treated with S4As, S6As, S8As

Day	Body weight (g) Mean/group $\pm$ SD (N = 10 animals/group)							
	1	3	5	7	9	11	13	14
S4As	23 $\pm$	22 $\pm$	22 $\pm$	23 $\pm$	25 $\pm$	26 $\pm$	26 $\pm$	26 $\pm$
500 mg/kg bw	0.74	0.62	0.64	0.60	0.42	0.27	0.35	0.38
p/initial	-	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Effect %/ initial	-	-4.3	-4.3	0	+8.7	+13.04	+13.04	+13.04
S6 As	22 $\pm$	21 $\pm$	22 $\pm$	24 $\pm$	24 $\pm$	25 $\pm$	25 $\pm$	25 $\pm$
500 mg/kg bw	0.42	0.41	0.33	0.38	0.36	0.34	0.33	0.32
p/initial		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Effect %/ initial		-4.7	0	+9.09	+9.09	+13.6	+13.6	+13.6
S8 As	27 $\pm$	27 $\pm$	28 $\pm$	29 $\pm$	30 $\pm$	31 $\pm$	32 $\pm$	32 $\pm$
500 mg/kg bw	0.67	0.63	0.59	0.62	0.64	0.57	0.52	0.54
p/initial		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Effect %/ initial		0	+3.70	+7.40	+11.11	+14.81	+18.51	+18.51

Experimental results from the chemical stimulus test indicated that all investigated compounds exerted analgesic effect. The intensity of the

analgesic effect for the compounds S4As, S6As and S8As is greater (statistically significant) compared with the control group (table II).

Table II

Intensity of analgesic effect for compounds S4As, S6As, S8As compared to control group treated with distilled water and to reference group treated with acetylsalicylic acid

Compound/dose	No.of writhings mean/group $\pm$ SD	<i>p</i>	Analgesic effect %/control	Effect %/reference
Distilled water/ 0,1 mL/10 g p.o.	40 $\pm$ 1.9	-	-	-
Acetylsalicylic acid/ 100 mg/kg p.o.	29 $\pm$ 0.52	< 0.0001	27.5	-
S4As/ 250 mg/kg p.o.	31 $\pm$ 1.2	0.0013	22.5	-5
S6As/ 250 mg/kg p.o.	31 $\pm$ 0.70	0.0003	22.5	-5
S8As/ 250 mg/kg p.o.	25 $\pm$ 1.1	< 0.0001	37.5	+ 10

Analgesic effect %/control

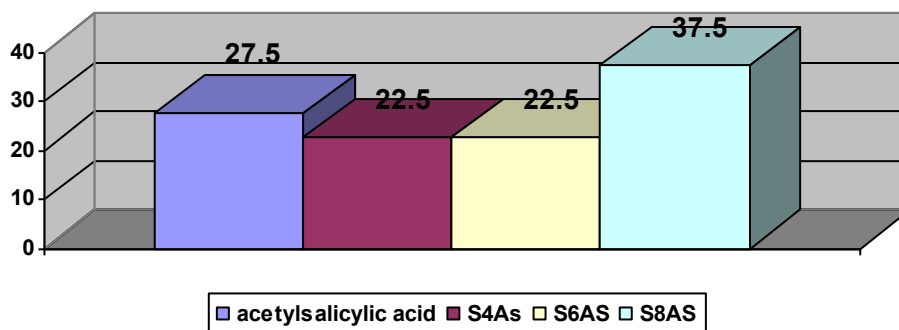


Figure 1.

Intensity of the analgesic effect (%) against the control group for the groups treated with acetylsalicylic acid as reference drug and investigated compounds S4As, S5As and S8As, in thermal stimulus test

The greatest intensity of the analgesic effect in the chemical stimulus test compared to control group treated with distilled water was recorded for S8As (37.5%). At the tested dose, 250 mg/kg-bw p.o., compound S8As exerts an intensity 10% greater than the reference substance, acetylsalicylic acid, administered in 100 mg/kg bw p.o. dose (figure no.1).

The experimental results from the hot-plate test indicated that all investigated compounds exerted analgesic activity, effect evidenced in the fact that the licking time in animals treated with S4As, S6As and S8As increases statistically significant as compared to the initial determination (table III).

Table III
Intensity of analgesic effect for acetylsalicylic acid and for investigated compounds: S4As, S6As, S8As

Substance/dose	Initially licking time (sec) $\pm$ SD	Licking time after 45 min (sec) $\pm$ SD	<i>p</i> /control	Effect %/initial	Analgesic effect %/control	Analgesic effect %/reference
Distilled water/ 0.1ml/10g bw p.o.	11 $\pm$ 1.1	11 $\pm$ 1.2		0		
Acetylsalicylic acid/100mg/kg bw p.o.	9.1 $\pm$ 0.97	18 $\pm$ 2.4	< 0.00001	97.80	97.80	
S4As/250 mg/kg bw p.o.	12 $\pm$ 1.5	20 $\pm$ 2.7	0.003	66.66	66.66	-31.14
S6As/250 mg/kg bw p.o.	8.5 $\pm$ 0.66	17 $\pm$ 2.5	< 0.00001	100	100	+ 2.2
S8As/250 mg/kg bw p.o.	8.6 $\pm$ 1.1	13 $\pm$ 1.5	0.005	51.16	51.16	-46.64

The analgesic effect against the control group is intense, as compound S6As induces 100% analgesia.

For all three investigated compounds the intensity of the analgesic effect (%) exceeds 50 against the control group (figure no.2). Compared to reference – acetylsalicylic acid, two compounds yielded a lower intensity of the analgesic effect, that is -31.14% for S4As and -46.64% for S8As. Compound S6As exerts slightly increased analgesic effect than acetylsalicylic acid (+2.2%).

Analgesic effect %/control

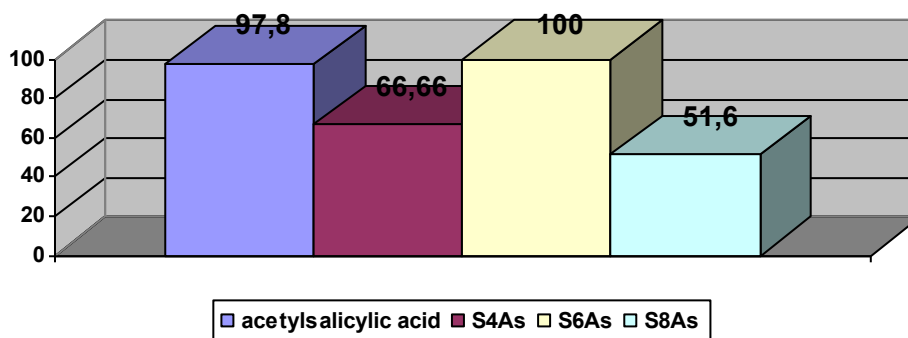


Figure 2.

Intensity of analgesic effect (%) against control group for the groups treated with reference – acetylsalicylic acid and with investigated compounds: S4As, S5As and S8As, in thermal stimulus test

Conclusions

Both tests used for demonstrating the analgesic activity of the three investigated compounds showed that the newly synthesized substances exert an intense analgesic effect compared to control group. Moreover, taking into account that the NSAID esterificated with the prostaglandinic derivative is the acetylsalicylic acid, the investigated substances may have a much lower gastro-intestinal toxicity compared to acetylsalicylic acid administered alone.

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Manuscript received: January 21st 2010