

THE STUDY OF THE ANTI-INFLAMMATORY ACTIVITY OF SOME THIAZOLYL- $\Delta_{2,1,3,4}$ OXADIAZOLES AND 5-CARBOXYETHYL-2-HYDRAZON-4-METHYL-THIAZOLE-DERIVATIVES

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Abstract

We evaluated the anti-inflammatory potential of some new thiazolyl-1,3,4-oxadiazolines and 5-carboxiethyl-2-hydrazon-4-methyl-thiazoles using an acute model of inflammation, by intramuscular administration of turpentine oil in which the response of the hematogen marrow (number of leukocytes, leukocitary formula and the degree of activation of the circulatory phagocytes) and the nitrate/nitrite levels, metabolites of NO have been determined. The experimental study was performed on rats. Four of the tested compounds showed a good anti-inflammatory potential, compared with diclofenac.

Rezumat

A fost studiată acțiunea antiinflamatoare a unor noi tiazolil-oxadiazoline și 5-carboxietil-2-hidrazon-4-metil-tiazoli, utilizând un model experimental de inflamație acută provocată prin injectare intramusculară a uleiului de terebentină la șobolani. S-a constatat că 4 dintre compușii testați prezintă o bună activitate antiinflamatoare, comparabilă cu cea a diclofenacului. Efectul antiinflamator s-a evidențiat prin reducerea modificărilor sistemice de fază acută (neutropenie, reducerea fagocitelor circulante și a activității fagocitare) precum și prin determinarea concentrației de nitrați/nitriți (metaboliți ai oxidului nitric).

Keywords: inflammation, thiazoles, oxadiazolines

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are an important part of today's therapy practice, with indications ranging from rheumatoid conditions to cardio-vascular, genito-urinary or stomatologic disorders. Considering that rheumatoid disorders have a prevalence of up to 7% of the world's population and therefore an important economic impact, due to increased therapy costs, it may be assumed that NSAIDs are a major

therapeutic class [8]. Although NSAIDs are a well represented pharmacological group, with numerous members, many of them are characterized by important adverse reactions, especially at the gastrointestinal level. That is why many of today's research efforts, in this field, are centered on obtaining new compounds with significantly reduced risks of adverse reactions.

Using as a starting point scientific literature that suggests the importance of the thiazole and oxadiazoline nuclei in the structure of known anti-inflammatory compounds [13,14], a study designed to assess the anti-inflammatory properties of a series of thiazolyl- Δ 2-1,3,4-oxadiazolines and 5-carboxyethyl-2-hydrazone-4-methyl-thiazoles in an acute inflammation model was conducted. The synthesis of these compounds was previously reported [4,5,6].

Due to the fact that nitric oxide (NO) is a key intermediate in the acute inflammatory process, the anti-inflammatory properties were also evaluated by monitoring the nitrate/nitrite levels, as metabolites of NO *via* the L-arginine-NO metabolic pathway [2,7].

Materials and methods

The acute model of inflammation used in this study consisted of intramuscular (i.m.) administration of turpentine oil to Wistar-Bratislava rats.

The anti-inflammatory potential of 12 newly synthesized compounds was tested (table I) using male albino Wistar rats. The animals, weighing between 200-250 g, were divided in 15 homogenous groups of 10 rats each.

At the same time 3 control groups were used: group **M** (untreated rats), group **I** (inflammation, only turpentine oil was administered) and group **D** (diclofenac was administered as a standard anti-inflammatory agent). Diclofenac was administered at a dose of 50 mg/kg b.w. (0.169 mmol/kg b.w.).

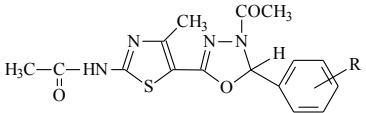
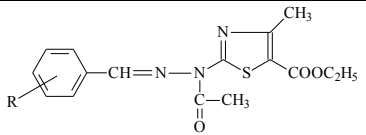
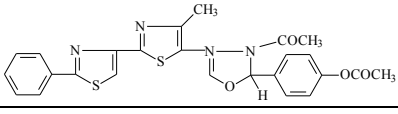
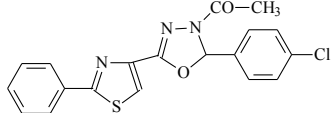
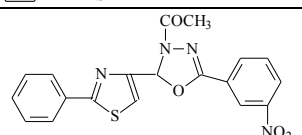
The investigated compounds were administered intraperitoneally (i.p.) as suspensions, using sodium carboxymethyl cellulose as suspension vehicle. The compounds were administered in the same molar concentration with the dose of diclofenac.

After 24 hours, blood samples were collected from the retro-orbital venous sinus of the rats from all 15 groups, and were analyzed by performing: a white blood cells count, a white blood cells differential count, an "*in vitro*" phagocytosis test and an evaluation of the nitrate/nitrite levels (metabolites of NO) [9,10,11].

The results have been statistically analyzed using the ANOVA test in order to compare the result of the control group with the other groups and by calculating the average, the standard deviation, the standard error and the confidence interval [1,3,12]. The differences between groups were considerate significant for $p < 0.005$.

Table I

The chemical structures of the investigated compounds

No.	Administered compound	Dose /kg b.w. animal	Adm. method
M	-	-	-
I	Turpentine oil	0.6 mL	im
D	Diclofenac	50 mg	ip
			
1	R = p-Cl	59.26 mg	ip
2	R = p-NO ₂	61.07 mg	ip
3	R = p-OCOCH ₃	64.68 mg	ip
4	R = o-Cl	59.26 mg	ip
5	R = o-Br	66.41 mg	ip
			
6	R = p-Br	64.37 mg	ip
7	R = o-OCH ₃	56.67 mg	ip
8	R = p-F	54.79 mg	ip
9	R = o-OCOCH ₃	61.03 mg	ip
10		79.47mg	ip
11		60.06 mg	ip
12		61.70 mg	ip

Results and discussion

The results were interpreted by:

- Quantification of the acute bone marrow response: a white blood cells differential count conducted on a peripheral blood sample.

- “*In vitro*” phagocytosis test: the phagocytic capacity of free leukocytes has been evaluated by the percentage of leukocytes that phagocytized at least one germ (**phagocytic index** = PI) and by the numbers of bacterial cells phagocytized by 100 leukocytes (**phagocytic activity** = PA) [9].

The administration of turpentine oil induces an acute local inflammatory reaction accompanied by systemic alterations. In that regard, the white blood cells count increased (leukocytosis) for the groups that received the turpentine oil in comparison with the control group **M** (untreated). This alteration is due to the effects of the acute phase cytokines on the bone marrow.

Analysing the white blood cells differential count, the results revealed that the increase in the number of leukocytes is mainly due to an increase in the number of micro-phagocytes, the first cellular elements to be activated by the defense mechanism. After 24 hours the main increase was in the number of neutrophils, basophils and eosinophils, cells that are less active in phagocytosis. Another important alteration was the decrease in the number of monocytes (they are being retained at the local inflammation site) and lymphocytes (responsible for unspecialized phagocytosis) (table II).

Table II

The results of the antiinflammatory test

No	Leukocytes /mm ³	Neutrophils %	Monocytes %	Lymphocytes %	Phagocytic index (PI)	Phagocytic activity (PA)	NaNO ₂ mg/dL
M	4952.7±1200	55.6±4	7.6±2.2	37±4.4	26.4±3.4	64.8±2.8	0.002
I	13528±1400	69.5±2.5	8±1	22.2±1.8	49.4±14	120.6±4.4	0.066
D	6500±900	60±2	6.8±2	31.3±3.8	20.7±2	28.2±2.8	0.0088
1	5680±940	45±2.2	5±1	50±5	20±3	40±2	0.00416
2	13280±880	68±3	4±2	28±3	45±1	100±1	0.0028
3	8600±11050	50±2	7±1	41±4	25±2	60±2	0.00154
4	13000±1100	70±4	4±1.4	26±2	45±3	99.6±2.4	0.00184
5	11120±800	60.4±3	5±2	34.6±4.4	39.6±1.4	90±3	0.0015
6	9840±640	45±5	7±1.2	48±4	30±3	70±4	0.00226
7	13840±710	70±2	4±1	26±1	55±3	130±2	0.00138
8	17840±640	78±2	3±1	19±2	60±4	140±4	0.0013
9	18520±1110	74.8±2.4	3±2	22.2±1.8	60±2	130±3	0.00916
10	15820±628	70±2	4.2±1.6	25.4±1.6	60±4	140±4	0.00176
11	21560±920	80±3	3±1	17±3	70±4	140±4	0.0033
12	15640±1420	60±2	5±1	35±1	50±4	121.6±3.4	0.00196

The concentration of nitrates/nitrites is increased in group **I** (inflammation), reinforcing the proven fact that their levels can be used to monitor an inflammatory process [2,7].

The white blood cells count dropped significantly ($p < 0.001$) in the case of compounds **1**, **3**, **5** and **6** by comparison with the control group **I**. Other tested compounds produced a less significant reduction of the number of leukocytes, while compounds **8** and **9** led to a significant increase in the total number of leukocytes. (table 2).

By analyzing the white blood cells differential count, significant differences can be observed regarding the percentage of phagocytes (neutrophils, lymphocytes, monocytes) when comparing compounds **1**, **3**, **5** and **6** with the control group **I**. There was a significant reduction in the number of neutrophils ($p < 0.0001$) and in the number of monocytes ($p < 0.0001$) accompanied by a simultaneous increase in the number of lymphocytes ($p < 0.001$) (table 2).

Regarding the acute inflammatory process, the “*in vitro*” phagocytosis test showed that in the case of group **I** there was an increase in the phagocytic capacity of circulating phagocytes. After 24 hours a significant increase ($p < 0.001$) in both phagocytic index (**PI**) and phagocytic activity (**PA**) could be observed when comparing with control group **M**.

For the groups that were administered compounds **1**, **3**, **5** and **6** the “*in vitro*” phagocytosis test revealed a significant ($p < 0.001$) decrease in the phagocytic capacity manifested by the reduction of both parameters: the ability of leukocytes to activate themselves and the number of germs phagocytized by the leukocytes (table 2).

The concentration of nitrates/nitrites was significantly reduced ($p < 10^{-7}$) by all the compounds tested. This indicates that one of the anti-inflammatory mechanisms of these compounds is the blockage of the L-arginine-NO metabolic pathway with the subsequent inhibition of the NO synthesis (table 2).

The results of our investigations clearly indicate that some of the tested derivatives (compounds **1**, **3**, **5** and **6**) have an anti-inflammatory effect, significantly reducing the bone marrow's response to the inflammatory process ($p < 0.0001$). Compounds **1**, **3** and **5** belong to the series of 4-N-acetyl-2[2'-(acetyl-amino)-4'-methyl-5'-thiazolyl]-5-aryl- Δ^2 -1,3,4-oxadiazolines while compound **6** belongs to the 5-carboxyethyl-2-(N₁-acetyl-hydrazone)-4-methyl-thiazole series.

The results also show that diclofenac reduces the inflammatory process by reducing **PI** by 60% and **PA** by 80%.

For the tested derivatives, the anti-inflammatory activity proved to

be inferior or with equivalent potency to that of diclofenac. They reduced the **PI** by 20-60% and the **PA** by 20-70%.

The most potent derivative from the series of 4-N-acetyl-2-[2'-(acetyl-amino)-4'-methyl-5'-thiazolyl]-5-aryl- Δ_2 -1,3,4-oxadiazolines is the compound **1**. This derivative has a Cl substituent in the *para* position of the phenyl ring and is characterized by an anti-inflammatory action comparable with that of diclofenac: it reduces **PI** by 60% and **PA** by 70%. The replacement of the Cl substituent with an acetoxy moiety (as is the case of compound **3**) results in a minor decrease of the anti-inflammatory action, **PI** and **PA** reductions being of 50% in comparison with the control group **I**. A substitution of the same position with NO₂ (compound **2**) led to a major loss of activity (no significant reduction of **PI** or **PA** takes place). The same lack of activity is obtained by moving the Cl substituent from the *para* to the *ortho* position of the phenyl ring (compound **4**). The presence of a Br substituent in the *ortho* position of the phenyl ring (compound **5**) results in a modest anti-inflammatory effect: **PI** is reduced by 20% and **PA** by 25%.

The only active compound from the series of 5-carboxyethyl-2-(N₁-acetyl-hydrazone)-4-methyl-thiazole is the compound **6**. This derivative has a Br substituent in the *para* position of the phenyl ring and has a good anti-inflammatory effect: **PI** and **PA** are reduced by 40%. Substitution with F in the *para* position of the same ring or substitution with the acetoxy or methoxy moieties in the *ortho* position led to a total loss of activity as is the case of compounds **7**, **8**, **9**.

The compounds from the 4-N-acetyl-5-R-2-(2''-phenyl-4'-methyl-2',4''-dithiazol-5'-il)-1,3,4- Δ_2 oxadiazoline (**10**), 4-N-acetyl-5-R-2-(2'-p-X-phenyl-thiazol-4'-il)- Δ_2 -1,3,4-oxadiazoline (**11**) and 4-N-acetyl-2-R-5-(2'-p-X-phenyl-thiazol-4'-il)- Δ_2 -1,3,4-oxadiazoline (**12**) series have not demonstrated any anti-inflammatory activity, moreover they showed an increase in **PI** and **PA** that could suggest a potentially pro-inflammatory activity.

Conclusions

The acute local inflammation accompanied by acute systemic modifications (which are evident for group I) is significantly decreased for the animal groups receiving the compounds **1**, **3**, **5** and **6**. The anti-inflammatory effect of these compounds is manifested by the reduction in the number of neutrophils and monocytes accompanied by a simultaneous increase in the number of lymphocytes, by a decrease in the phagocytic capacity and by the reduction of the concentration of nitrates/nitrites.

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