EVALUATION OF THE ANTI-INFLAMMATORY POTENTIAL OF SOME POLYHETEROCYCLIC COMPOUNDS WITH THIAZOLE RING IN ACUTE INFLAMMATION MODELS. PART II. CELLULAR RESPONSE

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Abstract

The cellular anti-inflammatory effect of some polyheterocyclic compounds with thiazole ring was evaluated in an acute experimental inflammation model. The compounds had an anti-inflammatory effect as they decreased the white cells count, the percentage of neutrophils and the phagocyte index.

Keywords: thiazole, acute inflammation, white cells count, neutrophils, phagocyte index

Introduction

Inflammation is appreciated as general, nonspecific response to tissue injury in many diseases. Local changes include vascular and cellular responses. The cellular one consists of leukocytes migration and activation into the inflamed tissue. It is now widely appreciated that uncontrolled inflammation can lead to secondary tissue injury, chronic inflammation, scarring and fibrosis. In acute inflammation, polymorphonuclears (PMN) represent first line host defense. Controlled responses of PMN phagocytes include destroying invading microorganisms and clearing sites of debris and
apoptotic neutrophils (PMN). In an excessive host’s response, PMN-mediated tissue injury leads to irreversible organ damage and associated diseases that are a major public health concern and financial burden [7].

In inflammations, first there is a need of drugs therapy to treat the acute phase. When inflammation becomes chronic, we have to treat not just the periods of acute attacks, but also the chronic process. Therefore, discovery of new specific and selective anti-inflammatory drugs is an important objective.

It was previously shown that thiazole derivatives, 1,2,4-triazoles derivatives and acylthiosemicarbazides possess anti-inflammatory activity [3, 6, 8]. Besides, several thiazole derivatives were found to be potent antitumour agents [1]. In this context, the aim of the present study was to evaluate the effects of two series comprising 21 novel thiazolic and 1,2,4-triazolic compounds [9], on an experimental model of acute inflammation by their influence on the local cellular response. The assessment was performed using indirect tests, respectively total white cells count (WCC), differential PMN count expressed as percentage (PMN%) and in vitro phagocytosis test [5].

**Materials and Methods**

*The studied thiazole compounds are listed below [9].*

![Figure 1a](image_url)

The structure of the tested thiazole compounds
Figure 1b
The structure of the tested thyazole compounds

**Turpentine oil-induced rat acute inflammation**

Adult male Wistar Bratislava rats, divided in 24 groups (n=10, 200-220 g b.w.), 8-12 weeks of age, of both sexes, supplied by the Animal Breeding Facility of the University of Medicine and Pharmacy Cluj-Napoca, Romania, were used. During the experiment animals were housed in temperature-controlled rooms, with 12:12 hours light: dark cycle and received water and food *ad libitum*. Procedures and animal treatments were conducted in accordance with the legislation governing experimentation on animals. The experiment was approved by the Ethics Committee. For all animals acute inflammation was induced through intramuscular injection with turpentine oil (0.6mL/100g body weight) [5].

The study groups were treated by i.p. injections as follows:

- an inflammation negative control group (INC) injected with the vehicle solution (distilled water and Tween 80) i.p. 1mL/animal
an anti-inflammatory control group treated with diclofenac (20mg/kg b. w.) i.p., 1 mL of solution/animal
21 test groups treated with new compounds (40mg/kg b.w.) i.p. as aqueous suspension in Tween 80, 1 mL of suspension/animal.

The dosed used was taken from published studies with thiazole compounds [10,11].

After 24 hours from inflammation induction, blood sample were collected from the retro orbital venous sinus in order to perform WCC, PMN% and in vitro phagocytosis test.

The WCC was performed with an optical microscope (Olympus), using a Bürcker-Türk counting-chamber. Differential leukocyte count expressed as a percentage was carried out on May-Grünwald-Giemsa stained smears [2].

In vitro phagocytes test was performed as previously described [5] with slight modifications. Blood samples were incubated in siliconated tubes with E.coli suspension at 37°C for 30 min, and smears stained with May-Grunwald Giemsa were examined with an optic microscope (Olympus). Phagocytic capacity was evaluated in terms of phagocytosis index (PI) (% of phagocytic cells in population with at least one phagocyted germ).

All results were expressed as mean ± standard deviation (SD) of 3 independent experiments. Statistical comparisons between the groups were made using one-way ANOVA test. A value of \( p<0.05 \) was considered to be statistically significant. Analysis was performed using standard software (SPSS for Windows, version 16.0).

Results and Discussion

The effect upon the WCC and PMN%

The obtained results clearly show that all substances decrease the acute phase medullar response as they significantly diminish the WCC (\( p<0.0001 \)). Moreover, the inhibitory effects of the new compounds were better than those of diclofenac (\( p<0.001 \)).

On the PMN%, the tested compounds had different effects. Compared to the negative inflammation group, substances 7, 9, 17, 19 lowered significantly the PMN%, making it even smaller than the PMN% of the group treated with diclofenac. Substances 4, 10-16, 18, 20-21 managed to decrease the PMN% similar to diclofenac (\( p<0.001 \)), whereas compounds 1, 2, 3, 5, 6 had a smaller inhibitory effect upon PMN% (\( p<0.05 \)) compared to diclofenac (Table I).

The effect upon the phagocytosis test

All tested compounds decreased the phagocyte index. Substances 4, 7-9, 11, 12, 14 16 had an extremely significant inhibiting effect (\( p<0.001 \)) which
was stronger than diclofenac’s effect. Compounds 3 and 10 acted similar to
diclofenac as they notably decreased the phagocyte index (p<0.001).
Substances 1, 2, 5, 6, 13, 15, 17-21 had a smaller decreasing impact than
diclofenac but moderately significant (p<0.05) when compared to the
inflammation group (Table I).

Rapidly recruited to the inflammatory site, PMN exert a variety of
primarily beneficial functions, such as phagocytosis, production of reactive
oxygen species, and nitric oxide species, and degranulation of lytic
enzymes. When well orchestrated, these processes enable clearance of the
invading pathogen. However, it is also hypothesized that activated PMN
may possess harmful potential when these same functions are directed at
otherwise normal host tissue, culminating in injury and organ damage [4].

In inflammation the blood contains more PMN compared with other
normal vascular beds. The degree of neutrophilia has been correlated with
prognosis in inflammatory processes. That makes to appreciate that the
reduction in WCC and PMN% was a positive effect of the tested compound.

Table I
The acute phase medullar response within acute inflammation model induced by
intramuscular administration of turpentine and the phagocyte index

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose mg/kg b.w.</th>
<th>Leucocytes /mm³</th>
<th>PMN %</th>
<th>Phagocyte index</th>
</tr>
</thead>
<tbody>
<tr>
<td>INC*</td>
<td>-</td>
<td>1334±200.5</td>
<td>70±3.1</td>
<td>50±8.2</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>20</td>
<td>6735±160.2</td>
<td>62.2±2.8</td>
<td>20.6±2.2</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>4620±142.1</td>
<td>81.6±4.2</td>
<td>30±3.4</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>5190±132.1</td>
<td>73.6±4.5</td>
<td>29±2.2</td>
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<tr>
<td>3</td>
<td>40</td>
<td>4970±128.3</td>
<td>84±4.8</td>
<td>19.6±1.8</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>5690±119.2</td>
<td>65.2±3.2</td>
<td>14.4±1.5</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>3700±105.8</td>
<td>78.4±2.8</td>
<td>25.8±2.2</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>4950±200.2</td>
<td>70±3.3</td>
<td>38±2.8</td>
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<tr>
<td>7</td>
<td>40</td>
<td>2600±111.8</td>
<td>44.2±2.8</td>
<td>14.1±1.2</td>
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<tr>
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<td>1620±140.5</td>
<td>77.6±4.1</td>
<td>14±1.3</td>
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<td>9</td>
<td>40</td>
<td>2760±150.2</td>
<td>41.2±2.1</td>
<td>12.4±1.2</td>
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<tr>
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<td>4000±120.7</td>
<td>54.5±3.2</td>
<td>22±2.8</td>
</tr>
<tr>
<td>11</td>
<td>40</td>
<td>2400±118.5</td>
<td>48.5±2.2</td>
<td>12.8±4.5</td>
</tr>
<tr>
<td>12</td>
<td>40</td>
<td>3850±118.8</td>
<td>60.1±3.2</td>
<td>18.7±5.2</td>
</tr>
<tr>
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<td>40</td>
<td>5400±121.5</td>
<td>62±3.1</td>
<td>42±3.2</td>
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<tr>
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<td>66±2.8</td>
<td>18±2.2</td>
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<td>15</td>
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<td>52±1.9</td>
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<td>54±2.7</td>
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<tr>
<td>17</td>
<td>40</td>
<td>4200±128.2</td>
<td>46±2.2</td>
<td>42±3.2</td>
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<tr>
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<td>58±2.5</td>
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<td>4200±119.6</td>
<td>68±3.2</td>
<td>30±2.7</td>
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</tbody>
</table>

*INC – inflammation negative control group, threated with the vehicle solution
Conclusions

According to the study results we concluded that the studied compounds possessed an anti-inflammatory effect on the inflammatory cellular response associated to the acute inflammation. Compounds 4, 7, 9, 10, 11, 12, 14, 16 possess the best anti-inflammatory action upon the inflammatory cellular response. Further research is needed in order to test the efficiency of thiazole compounds on inflammatory cellular response in chronic inflammation models too.

Acknowledgements

This work was supported by CNCSIS-UEFISCSU, project number PN II-IDEI code 1269-2008.

References

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*Manuscript received: December 19th 2011*