Effect of Cinnamic and Acrylic Acids’ Derivatives on Luminol-Enhanced Chemiluminescence of Neutrophils

Z. Czuba, W. Krol, S. Scheller, and J. Shani*

Department of Microbiology, Silesian School of Medicine, 41-808 Zabrze-Rokitnica, Poland, and *Department of Pharmacology, The Hebrew University School of Pharmacy, Jerusalem, Israel

Z. Naturforsch. 47c, 753–756 (1992); received March 25/May 25, 1992

Chemiluminescence, Luminol, Cinnamic Acid, Acrylic Acid, Neutrophils

Fourteen derivatives of cinnamic and acrylic acids were evaluated for their ability to modulate chemiluminescence, evoked by neutrophils that had been exposed to luminol and phorbol-myristate-acetate. Compounds with one or two hydroxyl groups on the phenyl ring demonstrated significant inhibition of the chemiluminescence, but this inhibition was diminished by methoxylation. Saturation of the double bond in the aliphatic chain of cinnamic acid at C6 – enhanced the chemiluminescence to a small degree. All three acrylic acid derivatives demonstrated a marked inhibition of the luminol chemiluminescence, indicating that characteristics of the heterocyclic ring is of utmost importance in this activity.

Introduction

Neutrophils are the first line of defense of the body against microorganisms. When they are activated; i.e., during phagocytosis or pinocytosis – their oxygen metabolism is enhanced, respiratory bursts are noticed, and light is emitted [1−3]. Oxygen absorbed by the neutrophils via the NADPH-oxidase system is then being reduced to form O$_2^-$, which together with other cellular radicals destruct the phagocytized microorganism [4, 5]. Excessive production of such oxygen metabolites and their secretion from the cell (instead of their being inactivated in it) – damage cellular structures that initiate pathological processes in living tissues [6, 7]. Some metabolites released by neutrophils are derivatives of the inflammatory agent arachidonic acid [8, 9].

Derivatives of cinnamic acid and acrylic acid comprise the basic moiety of many medically-active natural products, e.g. propolis [10]. They also comprise many active drugs, demonstrating anti-inflammatory [11], anti-thyrotrophic [12] and antiviral properties and prostaglandin-synthetase stimulators [13–15]. The major differences between the variety of these compounds are the number and location of their hydroxyl or methoxyl groups, the possible saturation of the double bond in the aliphatic chain of the cinnamic acid derivatives, and the variability of the heterocyclic group of the acrylic acid derivatives. As the mechanism of action of many of these drugs involve anti-oxidation, and in lieu of our long-lasting interest in the pharmacology and therapeutic effects of propolis components, we decided to test the fourteen compounds, listed in Table I, for their anti-oxidative effect. This was evaluated by quantitating the intensity by which these compounds suppress neutrophil-chemiluminescence in the presence of luminol and in response to phorbol-myristate-acetate (PMA). PMA generates oxygen metabolism and chemiluminescence or neutrophils in the presence of luminol which is accompanied by emission of light.

Materials and Methods

Chemicals

Caffeic acid, 3,4-dimethoxycinnamic acid, isoferulic acid and Hanks buffer (HBSS) (without phenol red) were purchased from Serva Fine Chem. (Heidelberg, Germany). Luminol (hydrazide of 5-aminophtalic acid) was bought from LKB Wallac (Turku, Finland) and 3-(2-thienyl)-acrylic acid from Aldrich Chem. Co. (Milwaukee, W.I., U.S.A.). Ferulic acid, o-, m- and p-coumaric acids, cinnamic acid, dihydrocinnamic acid, dihydrocaffecic acid, acrylic acid, 3-(2-furyl)-acrylic acid, 3-(4-imidazolyl)-acrylic acid, kit for myeloperoxidase detection, PMA and histopaque (densi-
ty 1.119 and 1.077 g/l) were purchased from Sigma Chem. Co. (St. Louis, M.O., U.S.A.). Dimethyl sulfoxide (DMSO) was purchased from BDH (Poole, England) and glycogen from a serum and vaccine producer in Cracow, Poland. All test compounds were dissolved in DMSO, and the final DMSO concentration in the test samples was 1% v/v.

Neutrophils

Male albino guinea pigs, 250–300 g BW, were used as donors for neutrophils. Twenty ml 0.1% glycogen in saline were injected into their peritoneal cavities, and 18 h later the peritoneal cavities were rinsed with 50 ml HBSS buffer containing 10 IU/ml heparin. The washings were collected and centrifuged in a two-step density gradient, with histopaque densities of 1.119 and 1.077 g/l. After purification, the vitality of the neutrophils was checked with trypan blue, which stained only the dead cells. The same staining was also used for evaluating the cytotoxic efficacy of the tested test-compounds on the neutrophils, used at a concentration of 100 μM. Neutrophils containing myeloperoxidase were identified by a modification of Hanker's method [16], using 1,7-phenylendiamine and catechol as non-cancerogenous substrates.

Chemiluminescence of neutrophils

Washed neutrophils (1 x 10⁶) were suspended in HBSS buffer pH 7.4 and mixed with 10 μl of each of the test compounds’ solutions or with 10 μl of DMSO as controls. Final concentration of DMSO was 1% v/v. Luminol solution was then added, giving a final concentration of 5.6 x 10⁻⁶ M, and the test-tubes were incubated at 37 °C. 5 min later, PMA solution was added, to yield a final concentration of 1.6 x 10⁻⁷ M, and chemiluminescence was measured for 15 min, as described earlier [17]. The final volume of each sample was 1.0 ml.

Results

The two-step density-gradient purification of the peritoneal guinea-pig neutrophils yielded more than 98% myeloperoxidase-positive cells, with viability of not less than 96.5%. Their viability was not diminished by any of the compounds, tested at a concentration of 100 μM, or by incubating the cells at 37 °C for 1 h.

Oxygen metabolism of the neutrophils was evaluated by luminol-enhanced chemiluminescence, after stimulation with PMA. In order to compare the activity of the various test-compounds, their percent of inhibition, as compared to controls, was calculated, using the following formula:

\[
\text{Inhibition} (\%) = \frac{N_c - N_t}{N_c} \times 100
\]

where \(N_c\) were the counts per 15 min of the control impulse, and \(N_t\) the counts per 15 min generated by a test compound. The inhibitory percentages of the 14 compounds are listed in Table I. Those values are reported for a final concentration of 10⁻⁴ M.

The chemiluminescence inhibition by the test-compounds is the highest when they contain an hydroxyl group in the para position to the side chain of the cinnamic acid, as compared to compounds having such a group in the ortho or meta positions, or having methoxylated groups. Dihydrogenation of the double bond in the side chain abolishes the inhibition of chemiluminescence altogether, and even tends to enhance PMA-mediated chemiluminescence. No such modulations were noticed in the control systems, when no luminol or PMA were added to the reaction test-tubes. Among the acrylic acid derivatives – 3-(4-imidazolyl)-acrylic acid was the most suppressive agent of the heterocyclic compounds tested.

Discussion

The chemiluminescence of neutrophils depends on the intrinsic cell level of myeloperoxidase, and can take place in various sites within the cell. The latter phenomena is activated by PMA, which activates protein kinase C [18] and a phagocytic process in the cell, which in turn enhances production of oxygen metabolites and activate transport of substances into the cell [14]. In activated neutrophils there is a marked increase in the intensity of the redox processes, which results (after the addition of luminol) in chemiluminescence. The major products of such redox reactions are metabolites of NADPH-oxidase and myeloperoxidase (mainly oxygen radicals and hydrogen peroxide), as well as arachidonic acid metabolites, e.g. prostaglandines and leukotrienes.

The effect of cinnamic acid derivatives on neu-
Table I. Chemical structures of the tested cinnamic and acrylic acids' derivatives, and the percent inhibition (± SD) of neutrophil chemiluminescence (n = 3–5).

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Chemical Structure</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>% Inhibition ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamic acid</td>
<td>CH=CH−COOH</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>21 ± 12</td>
</tr>
<tr>
<td>o-coumaric acid</td>
<td>CH=CH−COOH</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>98 ± 4</td>
</tr>
<tr>
<td>m-coumaric acid</td>
<td>CH=CH−COOH</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>16 ± 7</td>
</tr>
<tr>
<td>p-coumaric acid</td>
<td>CH=CH−COOH</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>81 ± 10</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>CH=CH−COOH</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>88 ± 11</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>CH=CH−COOH</td>
<td>H</td>
<td>OCH₃</td>
<td>OH</td>
<td>95 ± 6</td>
</tr>
<tr>
<td>Isoferulic acid</td>
<td>CH=CH−COOH</td>
<td>OH</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>77 ± 11</td>
</tr>
<tr>
<td>3,4-dimethoxycinnamic acid</td>
<td>CH=CH−COOH</td>
<td>H</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Dihydrocinnamic acid</td>
<td>CH₂−CH₂−COOH</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Dihydroferulic acid</td>
<td>CH₂−CH₂−COOH</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>−6 ± 1</td>
</tr>
<tr>
<td>Acrylic acid</td>
<td>H</td>
<td></td>
<td></td>
<td></td>
<td>12 ± 6</td>
</tr>
<tr>
<td>3-(2-furyl)-acrylic acid</td>
<td>2-furyl</td>
<td></td>
<td></td>
<td></td>
<td>27 ± 4</td>
</tr>
<tr>
<td>3-(4-imidazolyl)-acrylic acid</td>
<td>4-imidazolyl</td>
<td></td>
<td></td>
<td></td>
<td>57 ± 8</td>
</tr>
<tr>
<td>3-(2-thienyl)-acrylic acid</td>
<td>2-thienyl</td>
<td></td>
<td></td>
<td></td>
<td>12 ± 1</td>
</tr>
</tbody>
</table>

Neutrophil chemiluminescence depends to a large extent on their redox reactions and metabolism of arachidonic acid. Derivatives possessing the double bond in their side chain, and hydroxyl groups on their phenyl ring, are being easily oxidized to quinone-type compounds [19]. Similar oxidations occur with caffeic and gallic acids [20]. Subsequent oxidation of caffeic acid results with cleavage of the phenyl ring with creation of a carboxylic group, a process which is reasonably fast in alkaline pH [21].

The minor stimulation of chemiluminescence demonstrated by dihydrocinnamic and dihydrocaffeic acids results most probably from their lack of double bond, which makes them less susceptible to oxidation and from stimulation of prostaglandin synthetase [15]. Caffeic, ferulic and isoferulic acids, but not dimethoxy-cinnamic acid, stimulate this enzyme, due to their anti-oxidative property [15].

In summary, cinnamic acid derivatives seem to have a dual effect: inactivation of redox reactions on one hand, and participation in reactions involving arachidonic acid metabolism on the other. The latter reactions include reactions involving lipoxygenase, cyclo-oxygenase and prostaglandin synthetase. While the hydroxy-derivatives of cinnamic acid represent the first route, the dihydroxy-cinnamic acid derivatives is typical to the second mechanism. Further tests are necessary to validate the structure-activity relationship between chemiluminescence and these two large groups of biologically-active compounds.

Acknowledgements

The authors wish to thank Mrs. Bogumila Kurylo for her valuable technical assistance.