PHARMACOLOGY AND TOXICOLOGY

Study of Anti-Inflammatory Action of Aurothiomalate, an Inhibitor of NF-κB

Yu. P. Belsky, A. N. Ivanova, M. G. Danilets, N. V. Belska, A. A. Ligatcheva, E. G. Uchasova, and D. V. Reikhart

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 2, pp. 153-156, February, 2011
Original article submitted October 28, 2010

We compared the effects of NF-κB inhibitor aurothiomalate and voltaren on NO production by mouse macrophages *in vitro*, their ability to cause local edema at the site of injection, and their effect on carrageenan-induced inflammation. High concentrations of aurothiomalate reduced NO production, while in low concentrations both aurothiomalate and voltaren stimulated this process. When injected into mouse footpad, aurothiomalate in a dose >1 mM and voltaren in a dose >1.6 μM induce paw edema. Both compounds suppressed carrageenan-induced inflammation, but the efficacy of aurothiomalate 2-fold exceeded that of voltaren.

Key Words: *NF-κB; macrophage; nitric oxide carrageenan-induced inflammation*

Inflammation is a global physiological response of the organism to pathogens. Impaired inflammation underlies the pathogenesis of the vast majority of human diseases, which necessitates the search for new pharmacological targets of regulation, on the one hand, and new safe and effective drugs, on the other. In modern clinical practice, inflammation is controlled by either non-steroidal anti-inflammatory drugs or steroids (glucocorticoids).

Infectious and noninfectious inducers of inflammation increase the expression of genes of proinflammatory cytokines (IL-6, TNF-α, IL-1β, IL-2, GM-CSF, M-CSF, and G-CSF), their receptors (IL-2 receptor α-chain, T-cell receptor β-chain), chemokines attracting inflammatory cells to the sites of inflammation (IL-8, MIP-1α, eotaxin), enzymes producing inflammatory mediators (including inducible cyclooxygenase-2, inducible NO synthase (iNOS), 5-lipoxygenase, phospholipase A2), and adhesion molecules (E-selectin). Transcription factor NF-κB plays the key role in the transcription of proinflammatory factors such as TNF-α, IL-1α, cyclooxygenase-2, iNOS, [1,5]. It was shown that NF-κB regulates the expression of genes involved in inflammation, immune response, and apoptosis [16], as well as in the pathogenesis of several chronic inflammatory diseases, such as rheumatoid arthritis, atherosclerosis, chronic obstructive pulmonary disease, multiple sclerosis, inflammatory bowel disease, and ulcerative colitis [6].

Inflammation is a result of cooperation of many cells coordinated in time and space. Macrophages play a special role in inflammation providing its duration and quality. Macrophages produce microbicidal factors attracting other cells to the site of inflammation, regulate vascular tone at the site of inflammation, and phagocytize and destroy infectious agents. Macrophages largely determine the outcome of inflammation, namely, its completion, recovery of damaged tissue (induction of apoptosis of lymphocytes involved in inflammation, absorption of damaged tis-

Institute of Pharmacology, Russian Academy of Medical Sciences, Siberian Branch, Tomsk, Russia. ***Address for correspondence:*** belsky59@mail.ru. Yu. P. Belsky

0007-4888/11/1512-0190

sues, production of extracellular matrix components and vasculogenesis) [3,7,8]. According to modern ideas about the need for targeted therapy, only non-steroidal anti-inflammatory drugs have a selective target, cyclooxygenase-2. Cyclooxygenase responsible for prostaglandin synthesis is still the main target of pharmacological regulation of inflammation.

Thus, the search for new pharmacological targets for suppression of inflammatory responses is a reflection of modern view on molecular mechanisms of pathological processes and is important from both the theoretical and practical points of view.

The aim of this work was to study the effects of NF-κB inhibitor, sodium aurothiomalate (Calbiochem) on macrophages *in vitro* (the inflammatory status of macrophages was evaluated by NO production) and on the course of carrageenan-induced inflammation *in vivo*.

MATERIALS AND METHODS

The experiments were performed on 8-12-week-old male and female C57Bl/6 mice (conventional mouse strain obtained from Department of Experimental Biological Models, Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences). The animals were kept in a semi-barrier system with 12:12 h illumination regime and received pellets and boiled drinking water acidified with hydrochloric acid (pH 4-5).

Voltaren (for injections, Novartis Pharma AG) belonging to a group of non-steroidal anti-inflammatory drugs was used as the reference drug.

NO production by macrophages was assessed by the amount of nitrites. For isolation of macrophages, the peritoneal cavity of mice was washed with cold saline (0.9% NaCl, Medsintez plant). The cells were harvested, resuspended in RPMI-1640 (State Research Center of Virology and Biotechnology Vector) with 10% ECS (HyClone), 20 mM HEPES (Sigma), 0.05 mM 2-mercaptoethanol (Sigma), 50 μg/ml gentamicin (Sigma), and 2 mM L-glutamine (Sigma), placed in plastic Petri dishes ($1.5\text{-}2.0\times10^6$/ml), and cultured for 2 h (5% CO_2). Adherent cells were collected. Macrophages were placed in 96-well flat-bottom plates and cultured for 2 days, then the supernatant was collected and nitrite content was measured spectrophotometrically using Griess reagent (at 540 nm).

Proinflammatory activity of the test agents, namely voltaren and aurothiomalate, at the injection site was studied as follows. Saline in a volume of 0.05 ml was injected under the aponeurosis of a hind paw (control); the test agents in the same volume were injected in the other hind paw (experiment). Local inflammatory response was evaluated after 4 h. The magnitude of the inflammatory response was determined as the difference between the weight of the control and experimental paws (in mg).

For evaluation of the effects of the test agents on carrageenan-induced edema, voltaren or aurothiomalate were injected into both paws. Carrageenan (type 1, Sigma; 1% solution) was also injected into the experimental paw. The total volume of the mixture was 0.05 ml. The reaction was also evaluated after 4 h as described above.

Statistical processing of the results was performed using Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

It is known that iNOS is expressed in response to bacterial endotoxin and inflammatory cytokines by some types of cells (the main of which are macrophages and smooth muscle cells of blood vessels) [9] and plays an important role in the inflammatory response. Experiments on the model of carrageenan-induced inflammation showed that suppression of iNOS activity and expression exerted an inhibitory effect and alleviated paw edema [4,11].

Evaluation of the effects of voltaren and aurothiomalate on NO production by mouse macrophages showed (Table 1) that the effect depended on the concentration of agents. Voltaren in the highest concentration (25 μM) had no effect on this parameter. Decreas-

TABLE 1. Effect of Voltaren and Sodium Aurothiomalate *in vitro* on NO Production by Mouse Peritoneal Macrophages (*X±m*)

Drug concentration, μM	Concentration of nitrite, optical density units
Voltaren	
0 (control)	2.35±0.57
6.25	14.12±0.84*
12.50	5.95±0.88*
25.00	3.70±0.42
Aurothiomalate	
0 (control)	13.67±0.97
1	18.68±0.70*
5	26.26±1.99*
10	13.05±0.42
50	1.73±0.54*
100	1.88±1.05*

Note. Here and in Tables 2-4: *$p<0.05$ compared to the control.

TABLE 2. Induction of Inflammation at the Site of Voltaren and Sodium Aurothiomalate Injection (*X±m*)

Drug concentration, μM	Reaction of inflammation, mg
Voltaren	
0 (control)	5.00±1.24
0.2	5.75±1.05
0.4	4.50±1.45
0.8	7.38±0.80
1.6	8.88±2.01
3.2	15.63±1.68*
6.4	14.50±1.19*
12.8	15.25±1.60*
Aurothiomalate	
0 (control)	4.00±0.55
1	3.00±0.71
10	4.75±1.18
100	5.50±0.85
1000	5.38±1.61
5000	18.13±1.83*
10,000	21.88±2.31*

ing voltaren concentration to 12.5 and 6.25 μM led to an increase in NO production by 2.5 and 6 times, respectively. Aurothiomalate decreased NO production below the control levels (baseline) in high concentrations (50 and 100 μM), but stimulated it in low concentrations: by 1.4 times at a concentration of 1 μM and 2-fold at a concentration of 5 μM.

It is known that non-steroidal anti-inflammatory drug (NSAID) can cause an inflammatory reaction at the injection site [14]. Bearing in mind that at certain concentrations both agents stimulated macrophage activity by increasing NO production, we evaluated the capacity of voltaren and aurothiomalate to induce edema after injection into animal paw (Table 2). Voltaren in doses of 0.2, 0.4, 0.8 and 1.6 μM did not cause edema. Higher doses (3.2, 6.4, and 12.8 μM) induced edema, but its severity did not depend on voltaren dose. These features of voltaren activity can be related to its property to stimulate cyclooxygenase and formation of prostaglandins at the site of injection [14]. Aurothiomalate in doses of 1, 10, 100 and 1000 μM did not induce swelling, while high doses (5 and 10 mM) promoted edema development.

On the basis of these results we chose the doses that do not cause the inflammatory reaction for evaluation of the effect of aurothiomalate and voltaren on carrageenan-induced edema. Voltaren in doses of 0.4, 0.8 and 1.6 μM reduced edema severity by ~2 times (Table 3); aurothiomalate also inhibited inflammation in all used doses (25, 50, 100, 200 and 400 μM). Comparison of anti-inflammatory activity of aurothiomalate (10 and 100 μM) and voltaren (1.6 μM) showed (Table 4) that the effectiveness of aurothiomalate in a dose of 100 μM 2-fold exceeded that of voltaren.

Carrageenan-induced edema is a common model for studying anti-inflammatory agents, since it allows assessing the contribution of different mediators participating in inflammation. It was shown that 1 h after injection of carrageenan, production of inflammatory factors such as prostaglandins, oxygen radicals, NO, and TNF-α increased [2,10,12,13]. It is known that NF-κB plays a key role in the production of inflammatory mediators by the cell [1,5]. Thus, this nucleophilic

TABLE 3. Effect of Voltaren and Sodium Aurothiomalate on Carrageenan-Induced Edema (*X±m*)

Drug concentration, μM	Inflammatory reaction	
	severity of edema, mg	% of control
Voltaren		
0 (control)	34.50±3.52	100
0.4	19.50±3.52*	51
0.8	17.60±3.19*	59
1.6	20.25±2.72*	64
Aurothiomalate		
0 (control)	46.25±2.14	100
25	16.00±4.09*	35
50	15.60±2.29*	34
100	20.60±4.27*	45
200	20.60±5.56*	45
400	15.20±4.07*	33

TABLE 4. Comparison of Anti-Inflammatory Effects of Voltaren and Sodium Aurothiomalate on Carrageenan-Induced Edema (*X±m*)

Drug concentration, μM		Inflammatory reaction	
		severity of edema, mg	% of control
0 (control)		25.40±1.78	100
Voltaren 1.6		18.67±0.86*	73
	10	24.40±4.40	96
Aurothiomalate	100	9.40±1.99*	37

factor, in contrast to cyclooxygenase, regulates several effectors of inflammation. For example, it was shown that NF-κB inhibitor isolated from propolis suppressed carrageenan-induced paw edema in mice. The primary mechanism of anti-inflammatory activity is blockade of iNOS synthesis [15]. The anti-inflammatory activity of aurothiomalate was significantly higher than that of voltaren probably due to the fact that it inhibited the expression of not one, but many inflammatory factors. In conclusion, it should be noted that NF-κB as a pharmacological target is more preferable cyclooxygenase, and its inhibitor aurothiomalate is a promising substance for the development of anti-inflammatory drug.

REFERENCES

1. P. J. Barnes and M. Karin, *N. Eng. J. Med*., **336**, No. 15, 1066-1071 (1997).
2. M. Bucci, F. Roviezzo, I. Posadas, *et al.*, *Proc. Natl. Acad. Sci. USA*, **102**, No. 3, 904-908 (2005).
3. I. M. Corraliza, M. Modolell, E. Ferber, and G. Soler, *Biochim. Biophys. Acta*, **1334**, Nos. 2-4, 123-128 (1997).
4. J. F. Fracasso, R. L. Nunes-de-Souza, C. E. Teixeira, *et al.*, *Braz. J. Med. Biol. Res*., **29**, No. 11, 1543-1548 (1996).
5. M. S. Hayden and S. Ghosh, *Gen Develop*., **18**, No. 18, 2195-2224 (2004).
6. T. Lawrence, *Cold Spring Harb. Perspect. Biol*., **1**, No. 6, a001651, (2009).
7. P. Loke, M. G. Nair, J. Parkinson, *et al.*, *BMC Immunology*, **3**, 7 (2002).
8. M. Modolell, I. M. Corraliza, F. Link, *et al., Eur. J. Immunol*., **25**, No. 4, 1101-1104 (1995).
9. S. Moncada, R. M. Palmer, and E. A. Higgs, *Pharmacol. Rev*., **43**, No. 2, 109-142 (1991).
10. I. Posadas, M. Bucci, F. Roviezzo, *et al.*, *Br. J. Pharmacol.,* **142**, No. 2, 331-338 (2004).
11. I. Rioja, A. Ubeda, M. C. Terencio, *et al.,Eur. J. Pharmacol*., **397**, No. 1, 207-217 (2000).
12. A. C. Rocha, E. S. Fernandes, N. L. Quintao, *et al., Br. J. Pharmacol*., **148**, No. 5, 688-695 (2006).
13. D. Salvemini, Z. Q. Wang, P. S. Wyatt, *et al.*, *Br. J. Pharmacol*., **118**, No. 4, 829-838 (1996).
14. D. L. Simmons, R. M. Botting, P. M. Robertson, *et al.*, *Proc. Natl. Acad. Sci. USA*, **96**, No. 6, 3275-3280 (1999).
15. K. Tan-no, T. Nakajima, T. Shoji, *et al.*, *Biol. Pharm. Bull*., **29**, No. 1, 96-99 (2006).
16. R. Weil and A. Israel, *Curr. Opin. Immunol*., **16**, No. 3, 374-381 (2004).