Effect of NF-κB Inhibitor Aurothiomalate on Local Inflammation in Experimental Th1- and Th2-Type Immune Response

A. A. Ligacheva, A. N. Ivanova, Yu. P. Belsky, N. V. Belska, E. S. Trofimova, M. G. Danilets, and A. M. Dygai

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 4, pp. 460-463, April, 2012
Original article submitted December 28, 2011

We compared the effect of NF-κB inhibitor aurothiomalate and voltaren on local inflammation in different types of immune response. Both substances reduced edema caused by sheep erythrocytes (Th1-type immune response) and local immediate-type hypersensitivity response induced with ovalbumin (Th2-dependent response). The anti-inflammatory effects of aurothiomalate were similar to those of voltaren during Th1-type immune response.

Key Words: *NF-κB, aurothiomalate; local inflammation; sheep erythrocytes; ovalbumin*

Qualitative characteristics of the immune response, its severity and outcome under various pathological conditions are determined by T lymphocyte polarization (Th1 or Th2) and predominance of this or that cytokine spectrum [1]. Th1-type immune response was shown to provide protective cell-mediated immunity [3]. Pronounced, but abnormal Th1-type immune response develops in various autoimmune diseases [5]. Excessive Th2-type immune response determines the pathogenesis of allergies [10]. The state of the immune system in tumors is also characterized by Th2-type polarization. Th1 cells secrete IFN-γ, IL-2, and lymphotoxin (TNF-β), promote production of IgG_{2a} antibodies by B cells, and participate in delayed-type hypersensitivity (DTH) reactions and protection against intracellular pathogens. Th2-cells produce IL4, IL-5, and IL-10. They predominate in helminth invasions and allergies, promote generation of IgE-synthesizing B cells, and are essential for humoral immune response [1].

Thus, despite the fact that inflammation is a normal physiological reaction to injury or penetration of infectious agents, there is a special system suppressing inflammation in the body; dysfunction of this system can lead to systemic inflammatory response syndrome fraught with the risk of multiple organ insufficiency. In this case, proinflammatory cytokines and reactive oxygen and nitrogen species normally neutralizing infectious agents can produce toxic effects on cells and tissues.

For suppression of undesirable inflammatory responses, non-steroidal anti-inflammatory drugs (aspirin, ibuprofen, diclofenac, naproxen, ketoprofen) that have no selective targets and therefore producing a negative effect on the gastrointestinal tract, or glucocorticoids also producing serious side effects are currently used. In light of this, the search for new selective pharmacological agents with targeted action and new targets for anti-inflammatory therapy is an important task and promising trend in modern immunopharmacology.

One of the modern biomolecular approaches to inflammation control is suppression of NF-κB, the major factor providing transcription of proinflammatory mediators (IL-1, TNF-α, and cyclooxygenase 2) [2]. For this purpose, inhibitors of NF-κB-mediated signal transduction pathways are used (*e.g.* aurothiomalate).

Institute of Pharmacology, Siberian Division of the Russian Academy of Medical Sciences, Tomsk, Russia. ***Address for correspondence:*** vittelli@mail.ru. A. A. Ligacheva

0007-4888/12/1534-0472

It is known that gold-containing compounds (phosphine derivatives auranofin and triethylphosphine gold chloride; thiol-containing compounds aurothioglucose and aurothiomalate) are widely used in clinical practice for the treatment of rheumatoid arthritis [4].

Anti-inflammatory properties of aurothiomalate are mediated by inhibition of NF-κB signaling accompanied by reduced production of NO and prostaglandin E2 by rat peritoneal macrophages via inhibition of IκB kinase [7].

Aurothiomalate also produces an antimetastatic effect suppressing NF-κB and thereby inhibiting expression of cell adhesion molecules E-selectin, ICAM-1 and VCAM-1 that play a key role in tumor cell adhesion [14]. In addition, gold-containing preparations exhibit cytotoxic properties and antitumor activity [13]. Aurothiomalate is a potent and selective inhibitor of protein kinase C (PKC)-dependent signaling pathway in neoplastic processes *in vitro* and *in vivo* [6,13]. It was also shown that gold-containing compounds (*e.g.*, auranofin) shift the Th1/Th2 balance towards Th2-profile *in vivo* and *in vitro* [8].

Here we studied the effect of NF-κB inhibitor sodium aurothiomalate (Calbiochem) on sheep erythrocyte-induced delayed-type hypersensitivity (DTH; Th1-dependent response) and ovalbumin-induced local immediate-type hypersensitivity (Th2-dependent response).

MATERIALS AND METHODS

The experiments were performed on 8-12-week-old male and female C57Bl/6 and BALB/c mice obtained from Department of Experimental Biological Models, Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences) (quality class 1). The animals were maintained under conditions of a 12/12 light regime and partial barrier system and received pellets and boiled drinking water acidified with hydrochloric acid (pH 4-5).

Voltaren (Novartis Pharma AG, a preparation for injections), a non-steroidal anti- inflammatory drug, was used as the reference preparation.

The effect of voltaren or aurothiomalate on local inflammation of different types was studied in animals with experimentally induced Th1- and Th2-dependent immune response. Th1-dependent immune response in C57Bl/6 mice was induced by immunization with sheep erythrocytes (SE). The animals received single intraperitoneal injection of SE suspension (10^8 cells; EKOlab) in a volume of 0.2 ml saline (Medsintez). Control animals were injected with 0.2 ml saline. DTH (Th1-induced edema) was assessed on the 6th day after SE immunization.

For induction of Th2-dependent immune response, BALB/c mice received three subcutaneous injections of 100 μg ovalbumin and 5 mg aluminum hydroxide (Sigma) as an adjuvant in 0.1 ml saline into the thigh with 14-day intervals. Controls were injected with 0.1 ml saline. Local immediate hypersensitivity was evaluated on the 7th day after the last immunization.

Voltaren and aurothiomalate in 0.05 ml saline were injected under the hind paw aponeurosis. Equivalent amount of saline was injected into the control paw. For evaluation of Th1- and Th-2 response, 10^8 SE or 20 μg ovalbumin, respectively, in a total volume of 0.05 ml were additionally injected into the experimental paw. Local inflammatory response was evaluated after 24 h (Th1-response) or 4 h (Th2-response). The magnitude of the inflammatory response was determined as the difference between the weights of control and experimental paws (in mg).

Statistical processing of the results was performed using one-way ANOVA followed by a posteriori Dunnett test. The differences were considered significant at $p<0.05$.

RESULTS

Aurothiomalate inhibits LPS-induced production of proinflammatory cytokines IL-1 and TNF-α in cultured human monocytes and mouse macrophages. In rheumatoid arthritis characterized by excessive Th1-type immune response, aurothiomalate reduced accumulation of inflammatory monocytes and macrophages in the synovial membrane and significantly inhibited production of IL-1, IL-6 and TNF-α by these cells [15].

The study of the effects of aurothiomalate and voltaren on local inflammation of Th1-type (Table 1) showed that both substances decreased SE-induced edema. Voltaren (980 μM) 2.9-fold reduced edema in comparison with the control (to 5.4 mg). Aurothiomalate (10 and 100 μM) decreased the response by 1.5 and 1.8 times, respectively (to 10.2 and 8.6 mg). The effect of aurothiomalate did not differ from that of voltaren. The inhibitory effect of aurothiomalate

TABLE 1. Effect of Aurothiomalate and Voltaren on DTH Response Induced by SE ($X \pm m$)

Substance		Edema, mg
Saline (control)		15.60±1.44$^+$
Voltaren (980 μM)		5.40±1.17*
Aurothiomalate	10 μM	10.20±1.98*
	100 μM	8.60±0.87*

Note. Here and in Table 2: $p<0.05$ in comparison with *control, $^+$voltaren.

TABLE 2. Effect of Aurothiomalate and Voltaren on Local Reaction of Immediate Hypersensitivity Induced by Ovalbumin ($X \pm m$)

Substance		Magnitude of edema, mg
Saline (control)		109.60±3.61+
Voltaren (980 μM)		35.80±2.84*
Aurothiomalate	10 μM	57.60±7.61*+
	100 μM	76.60±3.54*+

on Th1-dependent inflammatory response is related to reduced activity of NF-κB kinase complex (IKK-alpha and IKK-beta) probably due to blockade of sulfhydryl groups of the enzyme [7].

Apart from NF-κB inhibition, aurothiomalate suppress intracellular signal transduction in Th2-type polarization. Aurothiomalate prevents interaction between the PB1-domain of PKC and adapter molecule Par6 and thereby blocks PKC-dependent activation of the intracellular protein Rac1, which is an important molecular target in PKC-dependent malignant transformation [11]. Selective action of aurothiomalate is determined by its interaction with a unique cysteine residue, Cys-69 localized within PKC PB1 domain, which prevents its interaction with arginine residue (Arg-28) of adapter molecule Par6 [6].

In light of this, we studied the effect of voltaren and aurothiomalate on local inflammation in experimental Th2-type immune response (Table 2). Both substances reduced ovalbumin-induced local immediate hypersensitivity response in comparison with the control. Injection of voltaren (980 μM) reduced edema by 3 times in comparison with the control (to 35.8 mg). Aurothiomalate (10 μM and 100 μM) also suppressed inflammation: edema decreased by 1.9 and 1.4 times, respectively (*i.e.* to 57.6 and 76.6 mg). However, the anti-inflammatory effect of aurothiomalate in both concentrations in this model was significantly lower than that of voltaren.

The anti-inflammatory effect of aurothiomalate in local immediate hypersensitivity was most likely determined by its effect on PKC-dependent signal transduction. It is known that Th2-type polarization in mice is associated with enhanced expression and activity of protein kinase isoform PKCζ, an important mediator of IL-4 signal transduction, that inhibits Jak1 activation and subsequent phosphorylation and nuclear translocation of transcription factor Stat6, a hallmark of Th2-type polarization. Inhibition of allergic reactions mucus production in mouse lungs and reduced concentrations of IL-4, IL-5, IL-13 in the bronchoalveolar lavage fluid were revealed in the model of ovalbumin-induced airway inflammation (model of allergic airway disease) in transgenic PKCζ-deficient mice [9].

At the same time, PKCζ activation was sufficient for IκB kinase stimulation, and NF-κB activation, and κB-dependent transcriptional activation [4]. Atypical PKC via adapter protein P62 interact with RIP and TRAF6, important components of TNF-α and IL-1 signaling [12] typical of Th1-type immune response. Thus, aurothiomalate in Th1-dependent immune response can modulate NF-κB activity in via several pathways. It blocks sulfhydryl groups of IKK and indirectly inhibits PKC signaling. This probably explains the fact that its anti-inflammatory effect in this type of response is higher than in Th2-type inflammation and is comparable with effect of voltaren.

In conclusion it should be noted that NF-κB is an important pharmacological target and aurothiomalate is a promising substance for the development of new anti-inflammatory agents.

REFERENCES

1. A. K. Abbas, K. M. Murphy, and A. Sher, *Nature*, **383**, 787-793 (1996).
2. P. J. Barnes and M. Karin, *N. Eng. J. Med*., **336**, No. 15, 1066-1071 (1997).
3. S. Bhowmick, R. Ravindran, and N. Ali, *Vaccine*, **25**, No. 35, 6544-6556 (2007).
4. M. T. Diaz-Meco, M. J. Lallena, A. Monjas, *et al*., *J. Biol. Chem*., **274**, No. 28, 19,606-19,612 (1999).
5. C. Ekerfelt, M. Andersson, A. Olausson, *et al*., *Clin. Exp. Immunol*., **150**, No. 1, 189-197 (2007).
6. E. Erdogan, T. Lamark, M. Stallings-Mann, *et al., J. Biol. Chem*., **281**, No. 38, 28,450-28,459 (2006).
7. K. I. Jeon, J. Y. Jeong, and D. M. Jue, *J. Immunol*., **164**, No. 11, 5981-5989 (2000).
8. T. S. Kim, B. Y. Kang, M. H. Lee, *et al*., *Br. J. Pharmacol*., **134**, No. 3, 571-578 (2001).
9. P. Martin, R. Villares, S. Rodriguez-Mascarenhas, *et al*., *Proc. Natl. Acad. Sci. USA*., **102**, No. 28, 9866-9871 (2005).
10. O. A. Nyan, G. E. Walraven, W. A. Banya, *et al*., *Clin. Exp. Allergy*, **31**, No. 11, 1672-1678 (2001).
11. R. P. Regala, C. Weems, L. Jamieson, *et al*., *J. Biol. Chem*., **280**, No. 35, 31,109-31,115 (2005).
12. L. Sanz, M. T. Diaz-Meco, H. Nakano, and J. Moscat, *EMBO J*., **19**, No. 7, 1576-1586 (2000).
13. M. Stallings-Mann, L. Jamieson, R. P. Regala, *et al*., *Cancer Res*., **66**, No. 3, 1767-1774 (2006).
14. K. Tozawa, N. Kawai, Y. Hayashi, *et al*., *Cancer Lett*., 196, No. 1, 93-100 (2003).
15. G. Yanni, M. Nabil, M. R. Farahat, *et al*., *Ann. Rheum. Dis*., **53**, No. 5, 315-322 (1994).