Effect of Ivermectin on Function of Liver Macrophages

A. V. Viktorov and V. A. Yurkiv

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 12, pp. 645-647, December, 2003
Original article submitted September 22, 2003

Ivermectin had no effect on phagocytic function of liver macrophages, but blocked LPS-induced secretion of tumor necrosis factor, NO, prostaglandin E_2, and increase of intracellular concentration of Ca^{2+}. These inhibitory effects were observed only in the presence of chlorine ions in the extracellular medium, which attested to involvement of glycine-dependent chlorine channels.

Key Words: *ivermectin; liver macrophages; lipopolysaccharide; phagocytosis*

Macrocyclic lactone ivermectin (22,23-dihydroavermectin B_1) is widely used as an active ingredient in veterinarian antiparasitic drugs and in the treatment of onchocerciasis in humans [3]. Biological activity of ivermectin is based on its interactions with chlorine channels: glutamate- [9], GABA- [1], and glycine-activated channels/receptors [7]. Glycine-dependent channels attract special interest, because they were found in many immunocompetent cells, for example, in resident macrophages of the liver (Kupffer cells) [2]. Dietary glycine produces a pronounced antiinflammatory effect due to blocking the activation of Kupffer cells, alveolar macrophages, lymphocytes, and neutrophils induced by gram-negative bacterial LPS (endotoxin) [12]. Kupffer cells form the largest pool of resident macrophages in mammals and play an important role in body defense by removing bacteria and various foreign substances from the blood flow. In response to LPS stimulation Kupffer cells generate a wide spectrum of physiologically active substances (inflammatory mediators) [8], which not only modulate function of the entire complex of liver cells, but also can damage other organs and tissues. Among these mediators are tumor necrosis factor (TNF-α), NO, and eicosanoids (primarily prostaglandin E_2). The effect of ivermectin on liver macrophages has never been studied.

Central Institute of Epidemiology, Ministry of Health of Russian Federation, Moscow. ***Address for correspondence:*** farm@newtech.ru. Viktorov A. V.

MATERIALS AND METHODS

Purified wild type *Salmonella typhimurium* LPS (Sigma) and ivermectin (Merck), fura-2AM fluorescent calcium indicator (Molecular Probes), cell culture medium (Gibco), and radioimmunoassay (RIA) kits for prostaglandin E_2 (Amersham) and ELISA kits for TNF-α detection (Genzyme) were used in the study.

Kupffer cells were isolated from Spraque-Dawley male rats (250-300 g) as described previously [10,11]. The purity of cell suspension was at least 90% (according to peroxidase staining, morphological study, and capture of latex particles); more than 95% isolated cells were viable (Trypan blue exclusion test). The cells were cultured in 24-well culture plastic plates at a density of 5×10^5/well in RPMI 1640 supplemented with 10% calf serum in a CO_2 (5%) incubator at 37°C for 48 h before the experiment. LPS (together with 5% rat serum) was added to the culture medium in a final concentration of 1 μg/ml. Stock ivermectin solution in ethanol (1 mg/ml, or 1.15 mM) was used. In experiments on elimination of chlorine ions from the culture medium chloride was substituted for gluconate 2 min before adding ivermectin. Phagocytosis was evaluated by capture of latex particles by cultured cells [5]. To this end, 0.05% latex suspension (1-μ particles, 0.5 ml) was added to the well. Free particles were washed after 15, 30, 60, and 120 min. The number of latex particles in the cell cytoplasm was counted under a phase contrast microscope (×400), 30 cells per well, 3 wells for each experiment. The concentration of free

0007-4888/03/1366-0569$25.00

intracellular calcium ions ($[Ca^{2+}]_i$) was measured using fura-2 fluorescent probe [10] after detachment of adhesive cells. The concentration of TNF-α in the culture medium was measured by the immunoadsorbent method [6]. NO secretion was evaluated by nitrate accumulation in the culture medium (its concentration was measured by colorimetry using Griss reaction [4]. The concentration of prostaglandins secreted by cultured cells was measured by high-performance liquid chromatography in combination with RIA, as described previously [11].

RESULTS

Ivermectin in concentrations of 0.3-3.0 μM little modulated phagocytosis, which was seen from the capture of latex particles by cultured Kupffer cell (Fig. 1). The increase in $[Ca^{2+}]_i$ is an obligatory condition for activation of many processes in Kupffer cells [8]. Ivermectin does not change the level of $[Ca^{2+}]_i$, but can block the increase in intracellular calcium concentration induced by subsequent addition of LPS (Fig. 2); this effect was observed only in the presence of chlorine ions in the external medium. It can be hypothesized that vermectin can "protect" Kupffer cells from stimulation by other agonists acting via mobilization of intracellular calcium.

Control Kupffer cells secrete only negligible amount of prostaglandin E_2 (Fig. 3), which does not change after addition of ivermectin (1 μM). Contrary to this, activation of Kupffer cells by LPS leads to a manyfold increase in prostaglandin E_2 concentration; this parameter reached the maximum by the 24th hour, which is in line with published data [8,11]. Preincubation with ivermectin considerably suppressed LPS-induced secretion of prostaglandin E_2. Replacement of chloride with gluconate in the external medium completely abolished this effect of ivermectin.

TNF-α is one of the best known peptide mediators secreted by Kupffer cells during inflammatory process [8]. The concentration of TNF-α increased 25-30 times by the 4th hour of LPS stimulation (Fig. 3) and then decreased. Preincubation with ivermectin led to an appreciable reduction of TNF-α secretion caused by further addition of LPS, while removal of chlorine ions from the culture medium before addition of ivermectin completely abolished the inhibitory effect of this drug.

Experiments also showed that LPS activation of Kupffer cells induced accumulation of NO, one of the most important chemically reactive intermediate compounds of nitrogen, in the culture medium (Fig. 3). The concentration of NO increased 6-8-fold and peaked 48 h after the start of endotoxin stimulation. Ivemectin markedly inhibited LPS-induced production of

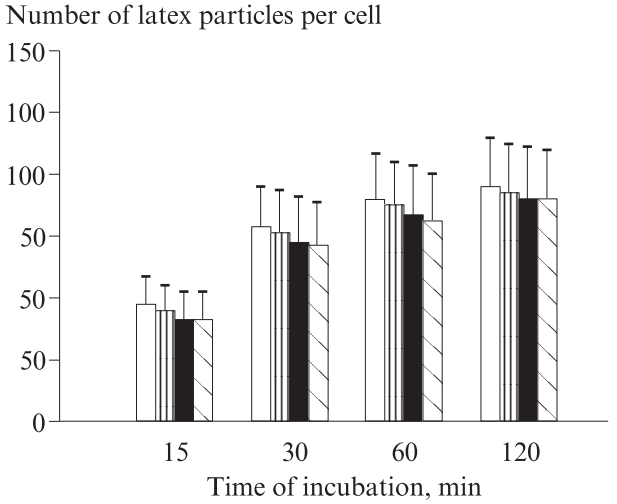

Fig. 1. Effects of ivermectin (IVM) in different concentrations on the capture of latex particles by cultured Kupffer cells. Light bars: control; vertically hatched bars: 0.3 μM IVM; dark bars: 1 μM IVM; cross-hatched bars: 3 μM IVM.

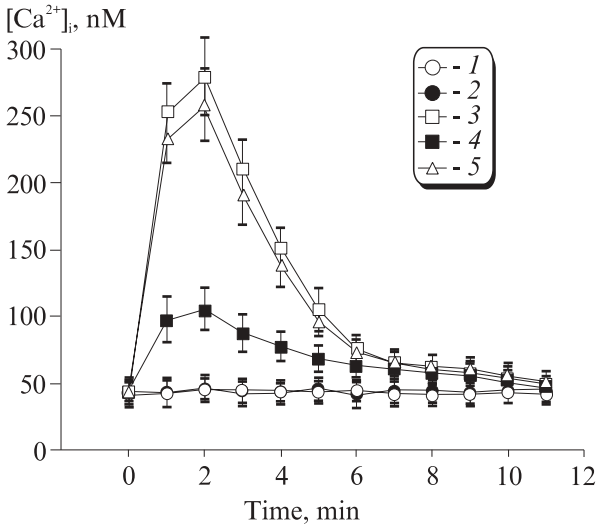

Fig. 2. Time dependence of the effect of 1 μM IVM on LPS-induced (1 μg/ml/5×10^5) increase $[Ca^{2+}]_i$ in suspension of Kupffer cells. Ivermectin was added 1 h before LPS (zero point). Removal of chlorine anions is shown as (-Cl). Here and in Fig. 3: *1*) control; *2*) IVM; *3*) LPS; *4*) IVM+LPS; *5*) -Cl+IVM+LPS.

NO, but only in the presence of chlorine ions in the medium.

The absolute dependence of the inactivating effect of ivermectin on the presence of chlorine ions in the medium is in line with published reports that ivermectin acts as an agonist of glycine-dependent chlorine channels expressed on liver macrophages [3,7]. The capacity of ivermectin to block LPS-induced secretion of macrophagal inflammatory mediators, on the one hand, and virtually not modulate phagocytosis, on the other, can have favorable physiological consequences at the organism level (reduction of symptoms of endotoxemia).

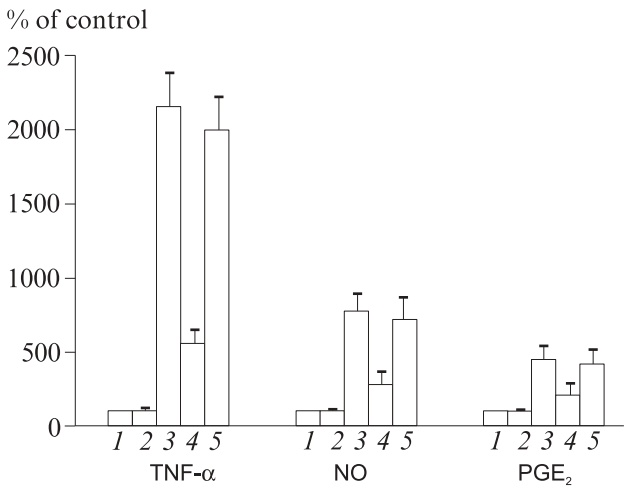

Fig. 3. Effect of 1 μM IVM on peak concentrations of TNF-α (4 h), NO (48 h), and prostaglandin E_2 (PGE_2; 24 h) secreted by primary culture of Kupffer cells under the effect of LPS (1 μg/ml/5×10^5 cells).

The authors are grateful to Professor D. Ernest (University of Arizona, Tyson, USA), and J. Hoek (Thomas Jefferson University, Philadelphia, USA) for assistance.

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