PHARMACOLOGY AND TOXICOLOGY

Albendazole and Colchicine Modulate LPS-Induced Secretion of Inflammatory Mediators by Liver Macrophages

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Colchicine and albendazole inhibited LPS-induced secretion of TNF-α and NO in a primary culture of rat Kupffer cells. Both agents potentiated the stimulating effect of this toxin on prostaglandin E_2 secretion. The amount of prostaglandin D_2 remained unchanged under these conditions.

Key Words: *albendazole; colchicine; liver macrophages; lipopolysaccharide; TNF-α*

Albendazole is a commonly used antihelminthic drug with a wide range of activities. This product is extensively used in medical and veterinary practice [4]. Similarly to other carbamate benzimidazoles, albendazole is characterized by high affinity for tubulin. Albendazole can cause dysfunction of the microtubular apparatus in cells [3]. The direct effect of albendazole on immunocompetent cells and, particularly, on macrophages is poorly understood. Colchicine has the same mechanism of action [3]. This drug produces an anti-inflammatory effect [5,6]. Similarly to albendazole, colchicine exhibits antihelminthic properties. Much attention was paid to studying of the effect of colchicine on macrophages. However, little is known about the influence of colchicine on LPS-induced secretion of secondary messengers by liver macrophages (Kupffer cells). LPS activates intracellular metabolic pathways that depend or do not depend on the microtubular structure of the cytoskeleton. Moreover, colchicine and albendazole exhibit antitumor (antiproliferative) properties that are directly related to the mechanism of their action [1,7].

Kupffer cells constitute the largest pool of resident macrophages in the mammalian body. They play a major role in the elimination of bacteria and foreign substances from the circulation. Kupffer cells produce a variety of physiologically active substances (mediators of the inflammatory process [9]) in response to stimulation with LPS. TNF-α, NO, and prostaglandin E_2 ($PG\text{-}E_2$) are of particular importance in this respect.

MATERIALS AND METHODS

Albendazole, colchicine, prostaglandin standards, and purified product of *Salmonella typhimurium* LPS (wild type) were purchased from Sigma.

Kupffer cells were isolated from male Sprague–Dawley rats (body weight 250 g) as described elsewhere [10,11]. The purity of Kupffer cells was at least 90% (peroxidase staining, morphology, and engulfment of latex particles). More than 95% isolated cells were stained by trypan blue (livable cells). The cells were cultured in 24-well plastic plates (5×10^5 cells/well) with RPMI 1640 medium containing 10% bovine

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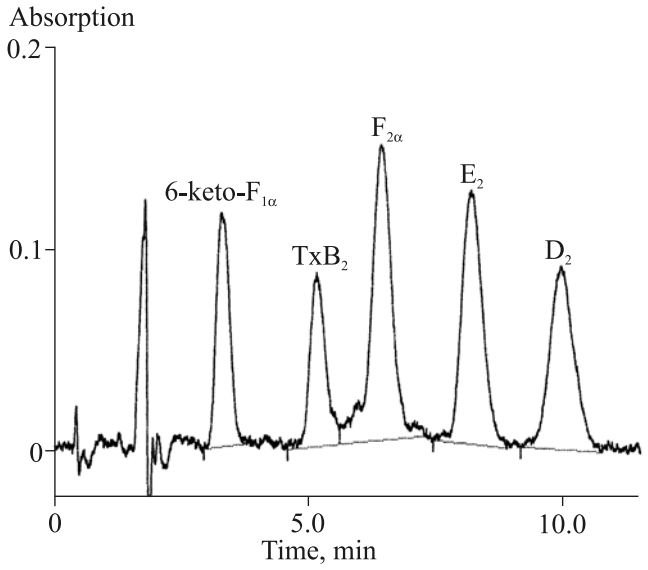

Fig. 1. HPLC chromatogram. Separation of PG standards (6-keto-$F_{1\alpha}$, TxB_2, $F_{2\alpha}$, E_2, and D_2). Injection of 2 μg of each PG. Detection of absorption at 194 nm. To estimate the concentrations of PG-E_2 and PG-D_2 in extracts from the culture liquid of Kupffer cells, we collected the fractions that coincided with the corresponding standards. The concentration of each PG in the fraction was measured by RIA as described elsewhere [11].

serum. Culturing was performed in an incubator at 37°C and 5% CO_2 for 48 h. LPS solution (in the presence of 5% rat serum) in a final concentration of 1 μg/ml was added to the culture medium. A stock solution of colchicine (100 mM, 39.9 mg/ml) in DMSO was stored at -20°C. A stock solution of albendazole (100 mM, 26.5 mg/ml) in ethanol was stored at 5°C. Preliminary experiments showed that addition of DMSO in this concentration to the cell culture has no effect on functional activity of cells. Kupffer cells were incubated with colchicine or albendazole in various concentrations for 3 h. Then these cells were stimulated with LPS. The concentrations of NO, TNF-α, PG-E_2, and PG-D_2 were measured in the culture medium. The concentrations of PG-E_2 and PG-D_2 were measured by HPLC and radioimmune assay (RIA; Amersham kits) as described elsewhere [11]. PG were extracted from the culture medium using SEP-PAK C_{18} cartridges and separated by the method of UV-HPLC on an analytical column C_{18} (Fig. 1). The fractions corresponding to PG standards were collected. The concentration of each component in these fractions was estimated by RIA. TNF-α concentration in the culture medium was measured by ELISA with Genzyme kits [8]. NO secretion was evaluated from nitrate accumulation in the culture medium. Nitrate concentration was measured colorimetrically by the Griess reaction [2].

RESULTS

Colchicine was potent in inhibiting the LPS-induced secretion of TNF-α by Kupffer cells, which is consistent with the results of previous studies on macrophages [6]. This property is probably a major cause of the anti-inflammatory effect of colchicine (Table 1). A decrease in TNF-α secretion became more pronounced with an increase in the concentration of colchicine and was maximum (-74%) at 100 μM. Similarly to colchicine, albendazole can prevent polymerization of tubulin. Under these experimental conditions, albendazole inhibited secretion of TNF-α. However, albendazole was less potent than colchicine in this respect (-47%).

Colchicine and albendazole inhibited LPS-induced secretion of NO by cultured Kupffer cells. The inhibitory effect depended directly on the concentration of this agents and was maximum at 100 μM. Albendazole was much more potent than colchicine (-42 and -60%, respectively).

Published data show that non-stimulated Kupffer cells mainly secrete PG-D_2 [11]. After LPS treatment, the concentration of PG-E_2 in the medium increases by 7-8 times and is comparable with that of PG-D_2. Colchicine had a strong stimulatory effect on PG-E_2 secretion (+51%), but did not modulate the basal level of PG-D_2. Albendazole produced a similar effect (+40%).

TABLE 1. Effects of Colchicine and Albendazole on LPS-Induced Secretion of NO, TNF-α, PG-E_2, and PG-D_2 by Kupffer Cells

Secondary messenger	Control	LPS	Colchicine+LPS				Albendazole+LPS			
			0.1 μM	1 μM	10 μM	100 μM	0.1 μM	1 μM	10 μM	100 μM
TNF-α, pg/ml	35±7	695±52	670±47	521±44	410±41	250±35	675±47	582±41	465±37	366±35
NO, μM	5.1±0.4	38±4	35±3	21±3	17±2	15±2	36±3	30±2	26±2	22±2
PG-E_2, ng/ml	0.6±0.1	4.5±0.4	4.8±0.4	5.4±0.4	6.3±0.5	6.8±0.5	4.6±0.4	5.1±0.5	5.9±0.5	6.3±0.5
PG-D_2, ng/ml	3.4±0.2	3.4±0.3	3.3±0.3	3.2±0.2	3.1±0.3	3.1±0.3	3.2±0.3	3.3±0.2	3.1±0.3	3.0±0.3

Note. Colchicine or albendazole was added 3 h before stimulation with LPS (1 μg/ml). The concentrations of TNF-α, NO, and PG were measured 4, 48, and 24 h after addition of LSP, respectively. This table shows the mean values of 3-4 experiments.

Our results suggest that LPS-induced secretion of TNF-α, NO, and PG-E_2 by Kupffer cells depends on functional activity of cytoskeletal microtubules. Cytoskeletal damage is accompanied by a significant decrease in this process. The mechanism of these changes remains unclear. This complex mechanism probably concerns various stages of the process (beginning from LPS binding to the cell).

It can be suggested that a commonly used antihelminthic drug albendazole produces the additional therapeutic effect in sepsis. This effect is related to inhibition of secretion of inflammatory mediators. These favorable properties should be taken into account when using albendazole and colchicine in antitumor therapy.

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