DOI 10.1007/s10517-021-05150-2

Antihistamines Modulate Functional Activity of Macrophages

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 170, No. 12, pp. 747-751, December, 2020
Original article submitted August 10, 2020

We compared the effects of the first-, second- and third-generation antihistamines in different doses on enzyme activity and cytokine production by macrophages and their death using an *in vitro* model. It was found that decreasing the dose led to an increase in the number of viable cells; after contact with second-generation antihistamines (loratadine, desloratadine), apoptosis of macrophages predominated. A dose-dependent increase in activity of ATPase and 5'-AMP with less pronounced effect of second-generation drugs was revealed. It was shown that under the influence of drugs, macrophages do not produce IL-1β, but actively synthesize TNFα and IL-10, which indicates the immunomodulatory properties of these drugs.

Key Words: *allergic diseases; antihistamines; macrophages; enzymes*

Histamine [2-(4-imidazolyl)ethylamine] is a potent inflammatory mediator affecting various populations of immune cells, including neutrophils and macrophages, by binding to 4 types of receptors: H1R, H2R, H3R, and H4R. It has been shown that histamine through binding to H2R stimulates the production of the chemoattractant protein MCP-1 and the expression of CCR2 receptors in monocytes [10]. Histamine via activation of H2R inhibits chemotaxis, phagocytosis, production of cytokines TNFα, IL-12, IL-27, and activity of NADPH oxidase, which plays a major role in generation of ROS in macrophages [8,9]. In general, these data demonstrate stimulating activity of histamine as an effector of the innate immune response, which further influences the development of inflammation in allergic diseases. These effects are abolished by first-, second-, and third-generation antihistamines; the latter are preferable for the treatment of allergic diseases, because they allow avoiding sedative and anticholinergic effects [4]. These drugs have anti-inflammatory properties (cetirizine, fexofenadine, loratadine, and desloratadine) due to blockade of histamine receptors and suppression of the expression of intercellular adhesion molecule-1 and cell chemotaxis. Some H1R antagonists have pleiotropic effects: they inhibit the release of leukotrienes, platelet activation factors, and other inflammatory mediators. Their effects are aimed at complex molecular mechanisms triggering activation of inflammatory cells, in particular, macrophages. It has been shown that histamine agonist-induced cross-desensitization and co-internalization of H1R and H2R receptors modulate the response of macrophages. Activation of H1R receptor affects the formation of cyclic AMP (cAMP) induced by histamine agonists via H2R receptors, while other drugs acting through H2R induce a negative regulation of the anti-inflammatory response by changing the expression of H1R [5]. In turn, H2R is associated with the G-protein and ensures the production of cAMP, activation of protein kinase A, and signaling to MAP-kinase, and also participates in phagocytosis of bacteria by macrophages [6]. It has been shown that H2R antagonist cimetidine eliminates histamine-mediated immunosuppression, because it has a powerful stimulating effect on the effector functions of neutrophils, monocytes, and macrophages [7]. On the other hand, there is a need to clarify the mechanism of antihistamines differing by their structure and effectiveness on innate immunity cells, the main participants in inflammation initiated by histamine.

The aim of the study was to compare the influence of antihistamines different generations on the death, enzyme activity, and cytokine production by macrophages.

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0007-4888/20/1706-0759

MATERIALS AND METHODS

We studied the effect of dexamethasone (Ellara) and antihistamines of the first (diphenhydramine, Belmedpreparaty), second (loratadine, Vertex; desloratadine, Teva Pharmaceutical Works Private), and the third generation (clemastine, Novartis Pharma Stein AG) on macrophages. The drugs were used in doses of 10, 5, and 2.5 μg/ml. Intact cells (not incubated with the drugs) served as the control.

The primary culture of peritoneal macrophages was obtained from outbred mice after induction of intraperitoneal inflammation (injection of 0.5 ml sterile 1% peptone water). In 24 h, the peritoneal cavity was washed and the obtained cell suspension was brought to a concentration of 2×10^6 cells/ml. All manipulations with animals were performed in compliance with the ethical principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasburg, 1986).

The studied preparations were added to the cell suspension, incubated for 30 min on a shaker (300 rpm) at 37°C, washed twice by centrifugation. Then the cells suspension (2×10^6 cells/ml) was transferred to 96-well plates and incubated in the medium containing 20% fetal calf serum (FCS), 2 μM L-glutamine (Merck), 0.004% gentamicin-K (Merck) for 2, 6, 18 h and 1, 2, and 4 days. Cell viability was assessed by measuring the concentration of formazan formed from 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (1 mg/ml, MTS, Merck) by the colorimetric method at 450 nm on a Multiskan Sky Microplate spectrophotometer (Thermo Fisher). To detect apoptosis and necrosis, the cells were incubated with 2 nM annexin V conjugated with fluorescein FITC (AnV) and 0.75-1.25 μg/ml propidium iodide (PI; BioLegend) for 20 min followed by analysis on a MACSQuant 6.0 flow cytometer (BioLegend); the number of AnV and PI-positive cells was determined in a two-parameter FL-1/FL-2 histogram using the Kaluza Analysis 2.1 software (Beckman Coulter). For evaluation of ATPase and 5'-AMP activities, the following substrates were used: Tris-HCl buffer (pH 7.8) containing 8 mg/ml ATP (Sigma-Aldrich) and 87 mg NaCl, 28.7 mg KCl, 52 mg $MgCl_2\times6\ H_2O$ and the same buffer containing 4 mg/ml AMP (Sigma-Aldrich), 87 mg NaCl, and 70 mg $MgCl_2$; the time of incubation was 30 and 60 min, respectively. The reaction was stopped by adding 100 μl mixture of ascorbic and molybdic acids (1:1) and the optical density of the solutions was measured at 620 nm. The content of proinflammatory (IL-1β, TNFα) and anti-inflammatory cytokines (IL-10) in culture supernatants was measured using Mouse ELISA Kit (Abcam) according to manufacturer's instruction. Optical density of the substrate (at 450 nm) was used to construct a calibration curve and determine the concentration of cytokines. The data from three independent experiments performed in triplicates are presented.

Statistical analysis of the results was performed using Statistica 8.0 (StatSoft, Inc.). All values are presented as $M\pm SEM$. Data for groups were analyzed using Student's t test and Newman—Keuls test for multiple comparisons at a confidence level of 95% or higher ($p<0.05$).

RESULTS

Evaluation of the ability of NADPH-dependent cellular mitochondrial oxidoreductase to oxidize MTS showed that dexamethasone exhibited maximum cytotoxicity towards macrophages. The number of viable cells increased with decreasing the dose (Fig. 1).

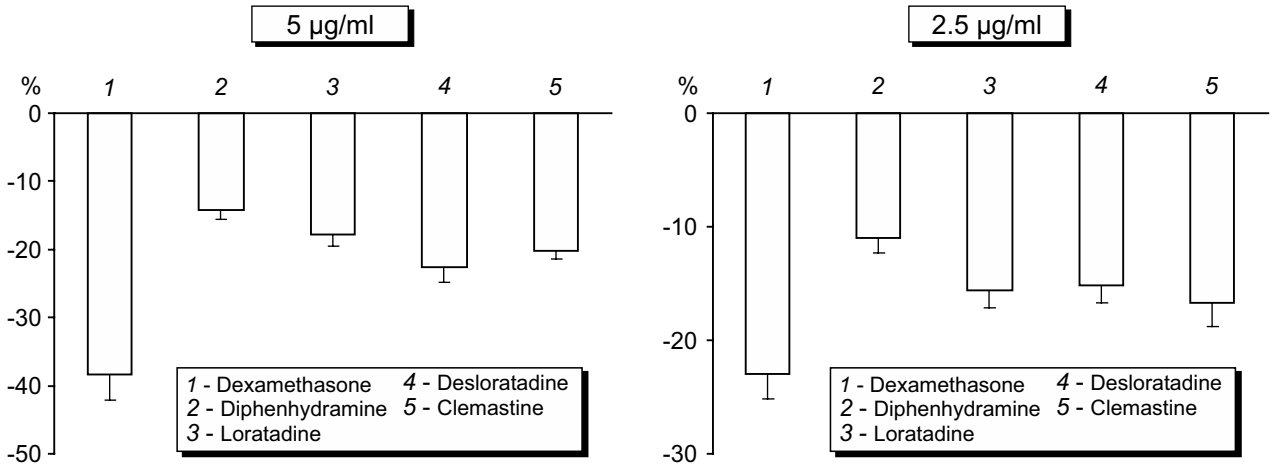

Fig. 1. Effect of dexamethasone and antihistamines on macrophage viability. Control was taken as 0%

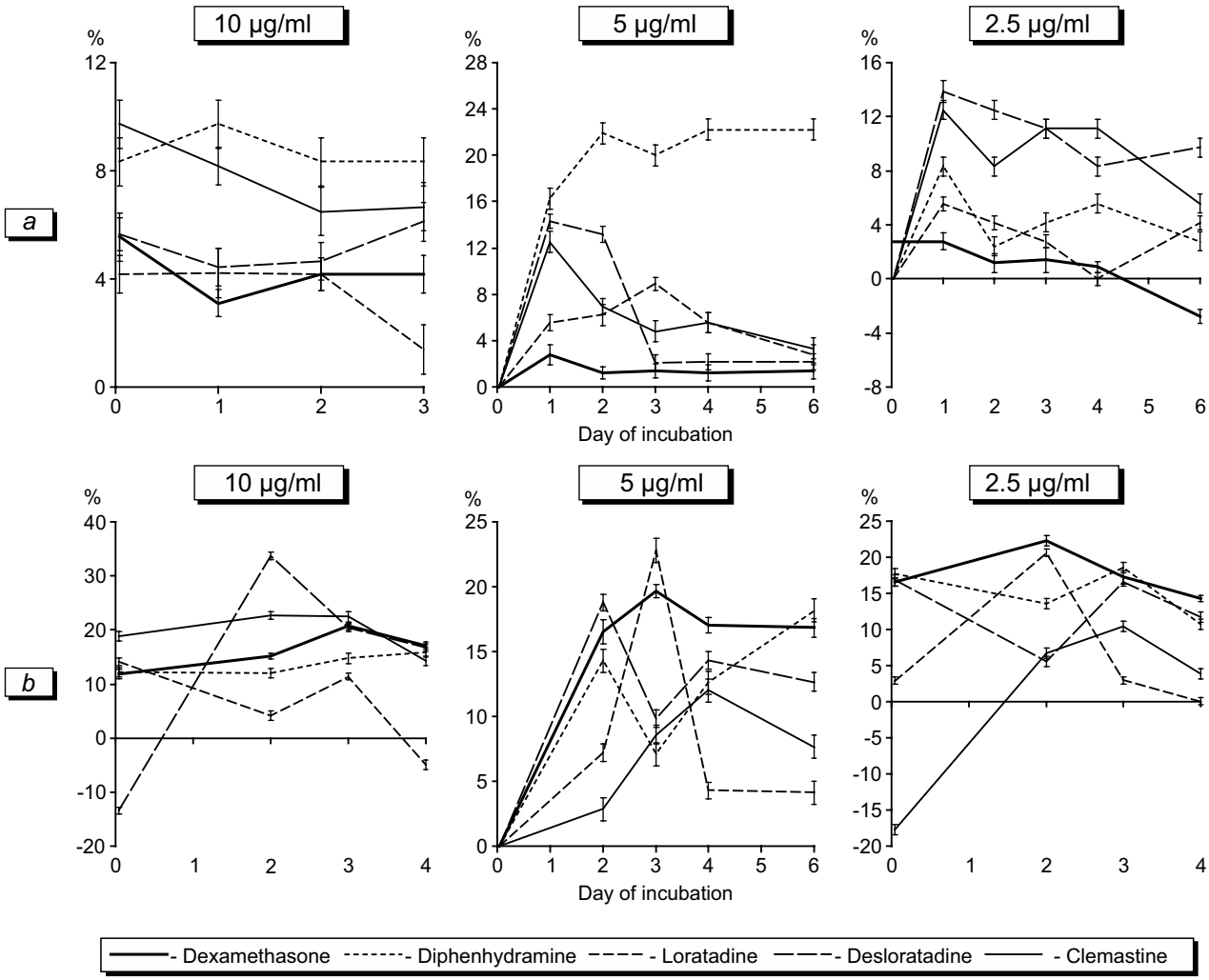

Fig. 2. Effect of dexamethasone and antihistamines on activity of ATPase (*a*) and 5'-AMP (*b*) in macrophages.

For antihistamines, the lowest cell viability was observed after contact with diphenhydramine in a dose of 2.5 μg/ml (p=0.01, Fig. 1).

Staining with AnV and flow cytometry showed that macrophages after contact with second-generation antihistamines predominantly died by apoptosis. For instance, the number of apoptotic cells after 4-day incubation in the control was 4.97±0.5%, while after contact with loratadine and desloratadine their number increased to 8.29±0.7 and 20.04±4.1%, respectively (drug dose 5 μg/ml). After incubation with the drugs in a dose of 10 μg/ml, the cells died mainly by necrosis and the maximum cell death was observed after contact with diphenhydramine (26.4±0.7%). Thus, the type of macrophage death depended on the type and applied dose of the drug.

Activity of 5'-AMP and ATPase is an indicator of ATP utilization by the cells. After cell contact with the test drugs, a dose-dependent increase in the content of these enzymes relative to intact cells was observed throughout the entire observed period (6 days) (Fig. 2, *a*). The increase in ATPase activity was observed after application of diphenhydramine in doses of 10 and 5 μg/ml and after application of desloratadine and clemastine in a dose of 2.5 μg/ml. The dynamics of intracellular 5'-AMP activity in macrophages slightly differed from that for ATPase (Fig. 2, *b*). The dose-dependence of 5'-AMP activity was revealed, but the maximum values after application of all doses were found for dexamethasone, diphenhydramine, and clemastine. In general, the above data demonstrate a pronounced stimulation of cells associated with the presence of synthetic activity, and a significant difference (p<0.05) between the indicators for various drugs for a smaller stimulating effect of second-generation antihistamines.

When analyzing the content of cytokines, we found that macrophages under the influence of the test drugs did not produce IL-1β, but actively synthesized TNFα. After application of all doses of dexa-

methasone, diphenhydramine, loratadine, and clemastine, we observed an increase in indicators from 221.74±13.7 pg/ml (5 μg/ml dexamethasone after 2 days) to 310.74±24.6 pg/ml (2.5 μg/ml diphenhydramine after 3 days) in comparison with intact cells (46.6±7.9 pg/ml; p=0.001). After incubation with desloratadine, these indicators were significantly lower: from 36.78±1.7 pg/ml (5 mg/ml after 4 days) to 81.46±4.8 pg/ml (2.5 mg/ml after 1 day), which attested to weak stimulating effect of this drug on macrophages. For anti-inflammatory cytokine IL 10, a significant (p=0.01) difference between the production of this cytokine was found in 6 days after contact with all drugs. The maximum values were observed after application of 5 μg/ml clemastine (247.2±14.6 pg/ml) and the minimum level was noted after application of same dose of loratadine (84.48±6.9 pg/ml). The value for intact cells was 25.63±3.7 pg/ml.

Biochemical and effector pathways in allergic diseases imply many potential targets and mechanisms for modulation of histamine receptor activity by a variety of drugs, *e.g.* suppression of the production of inflammatory cytokines and chemokines or stimulation of the migration and survival of inflammatory cells [1]. The results of our *in vitro* studies suggest that antihistamines produce a stimulating effect on macrophages. This effect can manifest itself in initiation of a more physiological type of death for these cells; in this case, the inflammatory response during an allergic reaction can be lesser pronounced [3]. On the other hand, the increase in ATPase and 5'-AMP activities associated with the presence of synthetic activity of macrophages indicates the ability of antihistamines to modulate some aspects of inflammation by the mechanisms other than H1R blockade. For instance, the increase in 5'-AMP activity is observed under conditions of ATP deficit, which leads to stimulation of AMP-activated protein kinase that phosphorylates acetyl-CoA carboxylase, thereby inhibiting fat synthesis [2]. Thus, at a low energy level, the protective reactions of the cell are activated, while the glucose storage mechanism is blocked. On the other hand, the dose-dependent effect on enzymatic activity of cells suggests that increasing the dose of antihistamines in allergic reactions can enhance the inflammatory response. Moreover, the increase in the production of cytokines by macrophages observed by us indicates their immunomodulatory properties. Despite more accurate targeting, the spectrum of effectiveness of second- and third-generation antihistamines for allergic diseases is much wider, *e.g.* high stimulating effect of clemastine on the production of TNFα and ATPase in macrophages obtained by us. Therefore, the possible molecular mechanisms of stimulation of activity of immune system cells require further study.

The study was supported by the program of intramural grants of the Pacific State Medical University, Ministry of Health of the Russian Federation.

REFERENCES

1. Prosekova EV, Turyanskaya AI, Sabynych VA. Assessment of interleukin-17 system in children with allergic bronchial asthma. Tikhookean. Med. Zh. 2018;(4):37-40. Russian.
2. Aschenbach WG, Sakamoto K, Goodyear LJ. 5' adenosine monophosphate-activated protein kinase, metabolism and exercise. Sports Med. 2004;34(2):91-103. doi: 10.2165/00007256-200434020-00003
3. Canonica GW, Blaiss M. Antihistaminic, anti-inflammatory, and antiallergic properties of the nonsedating second-generation antihistamine desloratadine: a review of the evidence. World Allergy Organ J. 2011;4(2):47-53. doi: 10.1097/WOX.0b013e3182093e19
4. Chazot PL. Histamine pharmacology: four years on. Br. J. Pharmacol. 2013;170(1):1-3. doi: 10.1111/bph.12319
5. Díaz Nebreda A, Zappia CD, Rodríguez González A, Sahores A, Sosa M, Burghi V, Monczor F, Davio C, Fernández N, Shayo C. Involvement of histamine H1 and H2 receptor inverse agonists in receptor's crossregulation. Eur. J. Pharmacol. 2019;847:42-52. doi: 10.1016/j.ejphar.2019.01.026
6. Fultz R, Engevik MA, Shi Z, Hall A, Herrmann B, Ganesh BP, Major A, Haag A, Mori-Akiyama Y, Versalovic J. Phagocytosis by macrophages depends on histamine H2 receptor signaling and scavenger receptor 1. Microbiologyopen. 2019;8(10):e908. doi: 10.1002/mbo3.908
7. Jafarzadeh A, Nemati M, Khorramdelazad H, Hassan ZM. Immunomodulatory properties of cimetidine: Its therapeutic potentials for treatment of immune-related diseases. Int. Immunopharmacol. 2019;70:156-166. doi: 10.1016/j.intimp.2019.02.026
8. Kmiecik T, Otocka-Kmiecik A, Górska-Ciebiada M, Ciebiada M. T lymphocytes as a target of histamine action. Arch. Med. Sci. 2012;8(1):154-161. doi: 10.5114/aoms.2012.27295
9. Mommert S, Aslan D, Ratz L, Stark H, Gutzmer R, Werfel T. The Anaphylatoxin C3a Receptor Expression on Human M2 Macrophages Is Down-Regulated by Stimulating the Histamine H4 Receptor and the IL-4 Receptor. J. Innate Immun. 2018;10(4):349-362. doi: 10.1159/000490426
10. Rocha SM, Pires J, Esteves M, Graça B, Bernardino L. Histamine: a new immunomodulatory player in the neuron-glia crosstalk. Front. Cell. Neurosci. 2014;8:120. doi: 10.3389/fncel.2014.00120