Effect of the Composition of Aluminum-Silica Carrier with Menthol, Camphor, Acidum Boricum, and Colloidal Silver on Monocytes Lines

Alexander Lykov
Research Institute of Clinical ans Experimental Lymphology-Branch of the Institute of Cytology and Genetics SB RAS;
Novosibirsk Tuberculosis Reseacrh Institute
Novosibirsk, Russia
aplykov2@mail.ru

Lubov Rachkovskaya
Research Institute of Clinical ans Experimental Lymphology-Branch of the Institute of Cytology and Genetics SB RAS
Novosibirsk, Russia
noolit@niikel. ru

Edmund Rachkovsky
Research Institute of Clinical ans Experimental Lymphology-Branch of the Institute of Cytology and Genetics SB RAS
Novosibirsk, Russia
reed@academ.org

Maria Surovtseva
Research Institute of Clinical ans Experimental Lymphology-Branch of the Institute of Cytology and Genetics SB RAS
Novosibirsk, Russia
mfelde@ngs.ru

Irina Kim
Research Institute of Clinical ans Experimental Lymphology-Branch of the Institute of Cytology and Genetics SB RAS
Novosibirsk, Russia
kii5@yandex.ru

Natalia Bondarenko
Research Institute of Clinical ans Experimental Lymphology-Branch of the Institute of Cytology and Genetics SB RAS
Novosibirsk, Russia
bond802888@yandex.ru

Olga Poveschenko
Research Institute of Clinical ans Experimental Lymphology-Branch of the Institute of Cytology and Genetics SB RAS
Novosibirsk, Russia
poveschenkoov@yandex

Abstract—**This work described effects of the composition of menthol, camphor, boric acid, and colloidal silver with particles of porous aluminum oxide with polydimethylsiloxane on THP-1 and U-937 cell lines *in vitro*. Drug carrier based on gamma aluminum oxide and polydimethylsiloxane composition (Al/PDMS) alone on in combination with menthol, camphor, boric acid and colloidal silver significantly increased NAD (P) H-dependent cellular oxidoreductase enzyme activity of THP-1 cells. While all tested samples decreased lactate production by THP-1 cells. Drug carrier alone or in combination with menthol, camphor, boric acid, and colloidal silver not affects glucose consumption and NO production by THP-1 cells. Drug carrier alone decreased U-937 cells NAD (P) H-dependent oxidoreductase enzyme activity, while in combination with active compounds significantly increased. We not obtained negative effect of all tested samples on lactate production by U-937 cells, but they decreased glucose consumption. It was estimated, that drug carrier alone increased NO production by U-937 cells.**

Keywords—Menthol, boric acid, aluminum oxide with polydimethylsiloxane, THP-1, U-937, MTT, lactate, glucose, NO

I. Introduction

The presence of menthol, camphor, boric acid and silver ions antimicrobial properties makes them attractive for the development of hygienic powders [1, 2]. Menthol is a local irritant, has an anti-inflammatory local effect, and antimicrobial activity is manifested by indiscriminate damage to microbial cells. Camphor has a cooling, irritating and partly antiseptic effect when applied topically. Boric acid often used as an antiseptic of wounds. Colloidal silver is a universal antibacterial agent with a wide spectrum of action.

In this report, we aimed to study the effect of the composition of aluminum-silica carriers with menthol, camphor, boric acid and colloidal silver on monocytes cell lines *in vitro*.

II. Material and Methods

The γ-Al_2O_3 particle with size 0.1 mm was modified by polydimethylsiloxane (Al/PDMS), and used as drug carrier [3]. Sorption of menthol (0.1%), camphor (0.1%), boric acid (0.1%), and colloidal silver (0.01%) was carried out in the aqueous phase at room temperature on Al/PDMS surface, followed by drying at a temperature of up to 50^{o} C.

Monocytes cell lines THP-1 and U-937 was cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 5 mM Hepes-buffer, and 1% of antibiotic/antimycotic in tissue flask, culture medium refreshed every 3-4 days. The 10^{6}/well of THP-1 or U-937 cells was sided into 24-well plate in culture medium, Al/PDMS, Al/PDMS/M/C/BA/Ag, menthol (M), and boric acid (BA) (0 and 1 mg/mL) was added into wells. Cytotoxicity of the used regents was done by MTT-test.

Lactate, glucose, and nitric oxide (NO) levels were measured in supernatants using spectrophotometer.

Statistical comparisons of obtained data were done by an ANOVA followed Bonferroni post hoc test. Data are expressed as means ± SD, differences were considered significant at $p \leq 0.05$.

III. Results

Obtained composition of menthol, camphor, boric acid, and colloidal silver with aluminum-silica carrier was bulk powder. In Table 1 summarized effects of menthol, boric acid, aluminum-silica carrier and composition of drug carrier with menthol, camphor, boric acid and colloidal silver on THP-1 cell line functional activities in *vitro*.

Menthol and boric acid alone decreased NAD (P) H-dependent cellular oxidoreductase enzymes activity of THP-1 cells compared with control ($p \leq 0.05$). While drug carrier and composition of aluminum-silica carrier with menthol, camphor, boric acid, and colloidal silver increased NAD (P) H-dependent cellular oxidoreductase enzymes activity of THP-1 cells compared with control ($p \leq 0.05$).

Aluminum-silica carrier, menthol, boric acid, and composition Al/PDMS/M/C/BA/Ag significantly decreased lactate production by THP-1 cells compared with control ($p \leq 0.05$).

When in culture medium present Al/PDMS, Al/PDMS/M/C/BA/Ag, and boric acid was observed decreased consumption glucose by THP-1 cells compared with control ($p \leq 0.05$). Whereas, THP-1 cells increased glucose consumption under menthol condition compared with control and Al/PDMS ($p \leq 0.05$).

We have no found significant changes of NO production by THP-1 cells in the presence of tested samples.

In Table II summarized effects of menthol, boric acid, aluminum-silica drug carrier, and composition Al/PDMS/M/C/BA/Ag on U-937 cell line functions. The NAD (P) H-dependent oxidoreductase enzyme activity was inhibited in the presence of aluminum-silica carrier, menthol, and boric acid compared with control group ($p \leq 0.05$).

TABLE I. EFFECT OF THE MENTHOL, BORIC ACID ALONE OR IN COMBINATION WITH ALUMINUM-SILICA CARRIER ON THP-1 CELL LINE PROPERTIES IN VITRO (M ± SD)

Parameters	Basal	Al/PDMS	Al/PDMS/M/C/BA/Ag	Menthol	Boric acid
MTT assay, OD	0.18±0.01	0.37±0.01*	0.29±0.01*#	0.13±0.01*#	0.15±0.01*#
Lactate, μM/L	1.53±0.1	1.33±0.1*	1.36±0.1*	0.71±0.1*#	0.89±0.1*
Glucose, μM/L	5.2±0.1	6.74±0.1*	6.62±0.1*	3.88±0.1*#	5.55±0.1*#
NO, μM/mL	4.65±0.2	5.0±0.2	4.6±0.2	4.9±0.2	4.65±0.2

Note. Al/PDMS, porous aluminum oxide with polydimethylsiloxane particles; Al/PDMS/M/C/BA/Ag; M, menthol; BA, boric acid; MTT assay tripartite; MTT assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide transformation into insoluble formazan by NAD(P)H-dependent cellular oxidoreductase enzymes in live cells; OD, optical density (λ=540 nm); NO, nitric oxide. *p≤0.05 compared with control; # p≤0.05 compared with Al/PDMS.

TABLE II. EFFECT OF THE MENTHOL, BORIC ACID ALONE OR IN COMBINATION WITH ALUMINUM-SILICA CARRIER ON U-937 CELL LINE PROPERTIES IN VITRO (M ± SD)

Parameters	Basal	Al/PDMS	Al/PDMS/M/C/BA/Ag	Menthol	Boric acid
MTT assay, OD	0.28±0.01	0.14±0.01*	0.43±0.01*#	0.16±0.01*#	0.22±0.01*#
Lactate, μM/L	2.26±0.1	2.27±0.1	2.43±0.1	6.07±0.1*#	5.63±0.1*#
Glucose, μM/L	2.26±0.1	3.6±0.1*	2.43±0.1#	6.07±0.1*#	5.63±0.1*#
NO, μM/mL	4.65±0.2	5.25±0.2*	4.85±0.2	5.7±0.2*#	5.35±0.2

Note. Al/PDMS, porous aluminum oxide with polydimethylsiloxane particles; Al/PDMS/M/C/BA/Ag; M, menthol; BA, boric acid; MTT assay tripartite; MTT assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide transformation into insoluble formazan by NAD(P)H-dependent cellular oxidoreductase enzymes in live cells; OD, optical density (λ=540 nm); NO, nitric oxide. *p≤0.05 compared with control; # p≤0.05 compared with Al/PDMS.

While composition Al/PDMS/M/C/BA/Ag possessed to increase NAD (P) H-dependent oxidoreductase enzyme activity of U-937 cells compared with control ($p \leq 0.05$).

Only menthol and boric acid alone significantly increased lactate production by U-937 cells compared with control ($p \leq 0.05$).

All tested samples, especially menthol and boric acid decreased glucose consumption by U-937 cells compared with control ($p \leq 0.05$).

In the presence of aluminum-silica carrier, menthol, and boric acid in culture media increased NO production by U-937 cells compared with control ($p \leq 0.05$).

IV. Disscussion

We have found that menthol, boric acid alone on in combination with aluminum-silica drug carrier possess to inhibit some enzyme intracellular activity in monocytes cell lines.

Menthol obtained from peppermint plant *Menthax piperita* belong to monoterpene. Menthol used by folk and traditional medicine for therapy of infections and as a non-opioid agent [4, 5].

Camphor obtained from *Cinnamomum campora* used in traditionally as anti-pain and anti-inflammatory activity [6, 7].

Boric acid is a weak chemical inorganic acid that has found its use as an insecticide, antiseptic. In addition, boric acid has an anti-inflammatory effect when applied externally [8].

Colloidal silver act as antimicrobial agent by deactivating of enzyme involved in bacterial respiration, proliferation and metabolism [9].

Cao and coworkers found that boric acid had no significantly affect THP-1 cells TNF-alpha production and intracellular glutathione content [10]. In rat model of gastric ulcer induced by ethanol methanol (50 mg/kg) caused gastroprotection, increased expression of cytoprotective and anti-apoptotic heat-shock protein-70, and decreased expression of apoptotic Bax protein in bowel [11]. Moreover, menthol treatment possessed to decrease myeloperoxidase and superoxide dismutase activity in

neutrophils, decreased TNF-alpha and IL-6 level, and increased IL-10 level.

Bayat and coworkers found dose-dependent inhibition of mononuclear cells proliferative activity, interferon-γ production, and reduced the number of CD+T cells expressing interferon-γ [12]. Under L-menthol human monocytes decreased production of LTB4, PGE2, and IL-1beta [13].

This is a first study to demonstrate the effects of the composition base menthol, camphor, boric acid, and colloidal silver with particle of γ-aluminum oxide with polydimethylsiloxane on monocytes cell lines *in vitro*.

REFERENCES

[1] O.V. Mosin, I. Ignatov, "Colloidal silver in bionanotechnology," Biotechnosphera, vol. 5-6, pp. 49-55, 2012.

[2] A.A. Kirilyuk, T.L. Petrishche, "Drugs applied for treatment of acute respiratory infections of the throat and cavity of the mouth: Pharmaceuticalassistance, assortiment and price availability in the repablic of Belarus (Part 1. Antiseptic medicines in the form of tablets for resorption)," Current problems of health care and medical statistics, vol. 3, pp. 92-105, 2017.

[3] A.P. Lykov, L.N. Rachkovskaya, O.V. Poveshchenko, M.A. Surovtseva, I.I. Kim, N.A. Bondarenko, E.E. Rachkovskii, M.A. Korolev, A.Y. Letyagin, "Cytotoxicity of aluminum-silica matrices modified with carbon nanotubes," Bull. Exp. Biol. Med., vol. 169(5), pp. 687-690, 2020.

[4] J.V. Jr. Pergolizzi, R. Jr. Taylor, J.A. LeQuang, R.B. Raffa; NEMA research Group, "The role and mechanism of action of menthol in topical analgesic products," J Clin Pharm Ther. , Vol. 43(3), pp. 313-319, 2018.

[5] J.A. Farco, O. Grundmann, "Menthol--pharmacology of an important naturally medicinal "cool"," Mini Rev Med Chem., vol. 13(1), pp. 124-131, 2013.

[6] N. Nchinech, A. Elgharbi, F.Z. Aglili, Y. Kriouile, Y. Cherrah, A.A. Mdaghri, S. Serragui, "Mésusage traditionnel du camphre: un danger oublié pour les enfants (à propos de 2 cas) [Traditional camphor misuse: a forgotten danger to children (about 2 cases)]," Pan Afr Med J., 2019. https:// doi.org/10.11604/pamj.2019.32.89.17943.

[7] S. Xiao, H. Yu, Y. Xie, Y. Guo, J. Fan, W, Yao, "The anti-inflammatory potential of Cinnamomum camphora (L.) J.Presl essential oil in vitro and in vivo," J Ethnopharmacol., 2021. https:// doi:.org/10.1016/j.jep.2020.113516.

[8] P. Ramos, M. Pieprzyca, B. Pilawa, " Effect of microwave power on shape of epr spectra--application to examination of complex free radical system in thermally sterilized acidum boricum," Acta Pol Pharm., vol. 73(2), pp. 291-296, 2016.

[9] A. Vila Domínguez, R. Ayerbe Algaba, A. Miro Canturri, Rodríguez A. Villodres, Y. Smani, "Antibacterial Activity of Colloidal Silver against Gram-Negative and Gram-Positive Bacteria," Antibiotics (Basel)., 2020. https://doi.org/10.3390/antibiotics9010036.

[10] J. Cao, L. Jiang, X. Zhang, X. Yao, C. Geng, X. Xue, L. Zhong, "Boric acid inhibits LPS-induced TNF-alpha formation through a thiol-dependent mechanisms in THP-1 cells," J. Trace Elem. Med. Biol., vol. 22(3), pp. 189-195, 2008.

[11] A.L. Rozza, F. Meira de Faria, A.R. Souza Brito, C.H. Pellizzon, "The gastroprotective effect of menthol: involment of anti-apoptotic, antioxidant and anti-inflammatory activities," PLoS One, 2014. https://doi.org/10.1371/journal.pone.0086686.

[12] M. Bayat, K. Kalantar, Z. Amirghofran, "Inhibition of interferon-γ production and T-bet expression by menthol treatment of human peripheral blood mononuclear cells," Immunopharmacol. Immunotoxicol., vol. 41(2), pp. 267-276, 2019.

[13] U.R. Juergens, M. Stober, H. Vetter, "The anti-inflammatory activity of L-menthol compared to mint oil in human monocytes in vitro: a novel perspective for its therapeutic use in inflammatory diseases," Eur. J. Med. Res., vol. 3(120, pp. 539-545, 1998.