[0336]

Effect of α-tocopherol on chemical mediators release from mast cells

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Background: It has been suggested that reactive oxygen species may play an important role in many diseases. In type I allergy such as hay fever, leukotrienes (LT) generated through the peroxidation of arachidonic acids by 5-lipoxygenase are responsible for the leukocyte migration. α-Tocopherol (Toc), a major lipophilic antioxidant in human body, has been implicated as an allergy inhibitor, however the mechanism is still unclear. In this study, we investigated effect of Toc on LT release from mast cells *in vitro*.

Method: Mouse mast cell line (PB-3c) pre-cultured with arachidonic acids for 48 h was used for LTB_4 release assay. Rat basophilic leukemia cell line (RBL-2H3) was used for assay of histamine, another chemical mediator (CM) released by the degranulation. The cells were incubated with Toc in Tyrode buffer for 10 min and then stimulated by calcium ionophore (A23187) for 20 min. Then the secreted LTB_4 and histamine were determined by HPLC/UV and HPLC/FL, respectively.

Results: LTB_4 released from PB-3c was inhibited by Toc at the physiological concentrations as in human serum. Histamine released from RBL-2H3 was also suppressed by Toc in a dose-dependent manner. On the other hand, tocopheryl quinone, oxidized form of Toc, showed no inhibitory effects on LTB_4 and histamine release. It suggests that antioxidant activity of Toc may be required for the suppression of CM release. Trolox, a hydrophilic analog of Toc, did not suppress the CM release either, meaning that the localization of Toc might affect the inhibition of CM release.

Conclusions: The results suggest that Toc may have the anti-allergic function through the suppression of CM release.

Keywords: tocopherol, allergy, leukotriene, mast cell

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[0405]

Polyphenols from Sardinian red wine can modulate NOX1-dependent reactive oxygen species production in human enterocyte-like cells treated with a dietary mixture of oxysterols

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Introduction: A large number of studies have suggested the beneficial role of polyphenols in human health, acting mainly as direct antioxidants. These compounds are widely distributed in all foods of plant origin, and their concentration varies according to different kinds of vegetable growth due to seasonal and geographic variations. Polyphenolic extract from Cannonau, a Sardinian red wine contains different types of polyphenolic compounds with antioxidant properties, particularly flavonoids and phenolic acids. In fact, dietary polyphenols have long residence time and accumulate in the mucosal intestinal layer, where they can exert their biological effects. We studied the role of the major polyphenols present in Cannonau on activation of NOX1 NADPH oxidase isoform, and reactive oxygen species (ROS) generation in differentiated CaCo-2 cells, a model for normal small intestine mucosa.

Methods: Differentiated CaCo-2 cells were treated with a mixture of the most common oxysterols found in cholesterol-rich foodstuff: 7-ketocholesterol, 5α,6α-epoxycholesterol, 5β,6β-epoxycholesterol, 7α-hydroxycholesterol, and 7β-hydroxycholesterol. Cells were pre-treated with Cannonau extract or with the singular major phenolic components [i.e. gallic acid, caffeic acid, (+)-catechin or (-)-epicatechin]. NOX1 activity was evaluated using Western Blotting (immunoprecipitating for the membrane enzyme component Nox1, and immunoblotting for cytosolic subunit NoxA1). ROS generation was visualized by laser confocal microscopy using 2',7'-dichlorofluorescein fluorescent probe.

Results: Oxysterols greatly interfere with the human digestive tract homeostasis, by promoting the damage of the colonic epithelial layer through NOX1 activation. Pre-treatment of oxysterol-challenged cells with Cannonau extract, in amount corresponding to one glass (100 ml) of wine, or with its major polyphenol compounds, diminished NOX1 activity and ROS generation.

Discussion: These findings suggests a strong antioxidant effect of phenolic compounds present in Cannonau, which could act not only directly as free-radical scavengers, but also indirectly by interfering with specific enzymes involved in redox cell signaling, such as NOX1.

Keywords: polyphenols, oxysterols, NADPH oxidase, CaCo-2 cells

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[0465]

Human Serum Albumin Masks DPPH Free Radical Sc avenging Potential of Dietary Polyphenols

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Polyphenols in plasma are bound to plasma proteins to some degree. The polyphenol-protein interaction (PPI) is reversible in that the polyphenol-protein complex can dissociate and release the free polyphenols. PPI is expected to modulate the bio-availability of polyphenols. Determining the influence of PPI on the antioxidant potential of polyphenols is critical and will directly correlate with the bioavailability of polyphenols. Herein, the influence of human serum albumin (HSA) on the DPPH free radical scavenging potential of polyphenols was investigated in detail. The antioxidant activities of polyphenols in the absence and presence of HSA were measured on the basis of the DPPH free radical scavenging activity.

Polyphenols scavenged DPPH free radical depending on their structure. Wogonoside, naringenin, naringin, silibinin, puerarin, biochanin A, soporicoside, genistein, daidzein, daidzin, genistin, galangin, hesperidin, and hesperitin showed very weak scavenging potential against DPPH free radical. However, kaempeferide, kaempferol, baicalin, myricetin, quercetin, luteolin, 7,8-dihydroxylflavone, fisetin, pyrogallic acid, myricetrin, bacalein, quercetrin, EGC, ECG, GCG, EGCG, rutin, dihydromyricetin, gallic acid, propryl gallate, ethyl gallate, and methyl gallate exhibited strong scavenging potential against DPPH free radical. HSA masked the DPPH radical scavenging potential of polyphenols. For example, DPPH free radical scavenging percentages of kaempferol and kaempeferide were 86.83% and 87.67% in the absence of HSA, respectively; however, free radical scavenging percentages reduced to 59.95% and 62.76% in the presence of HSA. The masking effect of these polyphenols with strong DPPH radical scavenging potential appears higher than those polyphenols with weak DPPH radical scavenging potential. The corresponding consequence of PPI is improving free polyphenols in blood, which causes fewer polyphenols expose to free radicals in blood. Therefore, polyphenols in blood are protected to be oxidized and can be efficiently delivered to other tissues, which enhances the beneficial impact of polyphenols.

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Keywords: Human Serum Albumin, DPPH Free Radical Scavenging, Dietary Polyphenols

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[0598]

Oxidative stress decreases glucose and neutral amino acid uptake in a human placental cell line (BeWo cells)

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Introduction and Aims: Increased oxidative stress (OS) has been implicated in the pathophysiology of gestational pathologies such as gestational diabetes, preeclampsia and hydatiform moles but its biomolecular targets and effects remain scarcely defined. So, our aim was to investigate the effect of OS upon the placental uptake of glucose and neutral amino acids (L-methionine (L-Met) and L-alanine (L-Ala)), which are nutrients necessary for an optimal human fetal development.

Methods: In a cell model of human trophoblasts, BeWo cells, elevated levels of OS were induced with 100 μM tert-butylhydroperoxide (tBOOH; 24h). ^{3}H-2-deoxyglucose (^{3}H-2-DG), ^{14}C-L-Met and ^{14}C-L-Ala uptake were assessed by liquid scintillometry.

Results and Discussion: Treatment of BeWo cells with tBOOH increased the levels of both total and oxidized glutathione, lipid peroxidation product malondialdehyde and protein carbonylation, confirming the induction of OS. These effects were not associated with changes in either BeWo cells viability or proliferation. In ^{3}H-2-DG transport assays, tBOOH reduced total and Na^{+}-independent ^{3}H-2-DG intracellular accumulation over time, by decreasing both facilitative glucose (GLUT)- and non-GLUT-mediated transport. Concerning ^{14}C-L-Met transport assays, our results demonstrated that tBOOH reduced total and Na^{+}-independent ^{14}C-L-Met intracellular accumulation over time. tBOOH did not affect system L-mediated but decreased non-system L-mediated ^{14}C-L-Met accumulation. Moreover, the kinetic parameters (K_m and V_{max}) of non-system L-mediated ^{14}C-L-Met uptake, which most probably corresponds to the low affinity and high capacity system y^{+}, were not significantly changed by tBOOH. Concerning ^{14}C-L-Ala uptake, tBOOH reduced total ^{14}C-L-Ala accumulation over time, through an inhibition of system A-mediated uptake.