FREE RADICAL PATHOPHYSIOLOGY

HEAD
ANTONÍN LOJEK

SCIENTISTS
MILAN ČÍŽ, LUKÁŠ KUBALA, IVANA PAPEŽÍKOVÁ, KATEŘINA PEJCHALOVÁ

TECHNICAL ASSISTANT
LENKA VYSTRČILOVÁ

GRADUATE STUDENTS
GABRIELA AMBROŽOVÁ, TOMÁŠ CRHÁK, MARTINA HAŠOVÁ, HANA KOLÁŘOVÁ, DANIELA KREJČOVÁ, LUCIE PRACHAŘOVÁ, MICHAELA PEKAROVÁ, MARTINA PODBORSKÁ, EMA RUSZOVÁ, ONDŘEJ VAŠÍČEK, LUCIE VIŠTEJNOVÁ

UNDERGRADUATE STUDENTS
LUCIA BINÓ, SILVIE GAJDOVÁ, HANA MARTIŠKOVÁ, EVA MATEJÍČKOVÁ, JANA NAVRÁTILOVÁ, MICHAL RÁJECKÝ, BARBORA ŠAFRÁNKOVÁ

The effects of H1-antihistamines on the nitric oxide production by RAW 264.7 cells with respect to their lipophilicity

H1-antihistamines are known to be important modulators of inflammatory response. However, the information about the influence of these drugs on reactive nitrogen species generation is still controversial. We investigated the effects of selected H1-antihistamines on nitric oxide production by lipopolysaccharide-stimulated murine macrophages RAW 264.7, measured as changes in inducible nitric oxide synthase (iNOS) protein expression in cell lysates by Western blotting and nitrite formation in cell supernatants using the Griess reaction. In pharmacological non-toxic concentrations, H1-antihistamines significantly inhibited nitrite accumulation that was not caused by the scavenging ability of drugs against nitric oxide, measured amperometrically. The degree of inhibition of nitrite accumulation positively correlated with the degree of tested lipophilicity, measured by reversed-phase thin layer chromatography. Furthermore, H1-antihistamines differentially modulated the iNOS protein expression. In conclusion, the modulation of nitric oxide production could be caused by the downregulation of iNOS protein expression and/or the iNOS protein activity.

Carvedilol and adrenergic agonists suppress the lipopolysaccharide-induced NO production in RAW 264.7 macrophages via the adrenergic receptors

The interaction of adrenergic agonists and/or antagonists with the adrenergic receptors expressed on immunologically active cells including macrophages plays an important role in regulation of inflammatory responses. We determined the effects of carvedilol, a unique vasodilating beta-adrenergic antagonist, and endogenous adrenergic agonists (adrenalin, noradrenalin, and dopamine) and/or antagonists (prazosin, atenolol) on lipopolysaccharide-stimulated nitric oxide (NO) production from murine macrophage cell line RAW 264.7. The production of NO was determined as the concentration of nitrites in cell supernatants (Griess reaction) and inducible nitric oxide synthase (iNOS) protein expression (Western blot analysis). Scavenging properties against NO were measured electrochemically. Carvedilol in a concentration range of 1, 5, 10 and 25 μM inhibited iNOS protein expression and decreased the nitrite concentration in cell supernatants. Adrenalin, noradrenalin or dopamine also inhibited the iNOS protein expression and the nitrite accumulation. Prazosine and atenolol prevented the effect of both carvedilol and adrenergic agonists on nitrite accumulation and iNOS expression in lipopolysaccharide-stimulated cells. These results, together with the absence of scavenging properties of carvedilol against NO, imply that both carvedilol and adrenergic agonists suppress the lipopolysaccharide-evoked NO production by macrophages through the activation and modulation of signaling pathways connected with adrenergic receptors.

GROUP OF PATHOPHYSIOLOGY OF FREE RADICALS IN CELL INTERACTIONS

GROUP LEADER
MILAN ČÍŽ

Oxidative modification of collagen influences breast cancer stem cell response to HNE

Breast cancer represents leading cause of mortality and morbidity in women, mostly due to property of primary tumor to metastasize. It was revealed recently that metastases comprise a fraction of stem-like cells, denoted as cancer stem cells (CSCs), usually located in the bone marrow. CSCs are of great importance in cancer biology as they are involved in blood vessel formation, promotion of cell motility and resistance to therapies and especially to metastasis development. One of the important factors influencing the stem cell destiny is their microenvironment and their interaction with extracellular matrix (ECM). Taking together the role of ECM in determining cell destiny and the involvement of lipids, lipid metabolism and lipid peroxidation in breast cancer development, we wanted to investigate the interactions between ECM and the growth regulating lipid peroxidation product 4-hydroxynonenal (HNE) on breast cancer stem cells. Our results indicate that oxidative modification of ECM collagen influences CSC growth, morphology and reaction to extracellular oxidative stress mediated by HNE and the growth inhibiting effects of this aldehyde. This is of importance as oxidative modification of ECM proteins could occur during local inflammation and during chemotherapies which cause lipid peroxidation. These modifications could be toxic for cancer and change gene expression, motility or stage of differentiation of malignant cells eventually maintaining oxidative homeostasis that could act against cancer.

Comparison of the antioxidant properties of vegetables using various methods

The present study investigates the antioxidant properties of selected vegetables, using the total peroxyl radical-trapping parameter (TRAP), oxygen radical absorbance capacity (ORAC) and hydroxyl radical averting capacity (HORAC) methods. ORAC, TRAP and HORAC values well correlated with polyphenol content. A good correlation was found also between the methods for measuring antioxidant capacity. Nevertheless, ORAC has been found to be the most sensitive method to measure chain breaking antioxidant activity. Although we have found a good correlation between TRAP, ORAC and HORAC, using more than one antioxidant assay is recommended for more detailed understanding the principles of antioxidant properties of samples.

GROUP OF FREE RADICALS IN REGULATION OF CELL PHYSIOLOGY

GROUP LEADER
LUKÁŠ KUBALA

Modulation of arachidonic and linoleic acid metabolites in myelo-peroxidase-deficient mice during acute inflammation

Acute inflammation is a common feature of many life-threatening pathologies, including septic shock. One hallmark of acute inflammation is the peroxidation of polyunsaturated fatty acids forming bioactive products that regulate inflammation. Myeloperoxidase (MPO) is an abundant phagocyte-derived hemoprotein released during phagocyte activation. Here, we investigated the role of MPO in modulating biologically active arachidonic acid (AA) and linoleic acid (LA) metabolites during acute inflammation. Wild-type and MPO-knockout (KO) mice were exposed to intraperitoneally injected endotoxin for 24 h, and plasma LA and AA oxidation products were comprehensively analyzed using a liquid chromatography-mass spectrometry method. Compared to wild-type mice, MPO-KO mice had significantly lower plasma levels of LA epoxides and corresponding LA- and AA-derived fatty acid diols. AA and LA hydroxy intermediates (hydroxyeicosatetraenoic and hydroxyoctadecadienoic acids) were also significantly lower in MPO-KO mice. Conversely, MPO-deficient mice had significantly higher plasma levels of cysteinyl-leukotrienes with well-known proinflammatory properties. In vitro experiments revealed significantly lower amounts of AA and LA epoxides, LA- and AA-derived fatty acid diols, and AA and LA hydroxy intermediates in stimulated polymorphonuclear neutrophils isolated from MPO-KO mice. Our results demonstrate that MPO modulates the balance of pro- and anti-inflammatory lipid mediators during acute inflammation and, in this way, may control acute inflammatory diseases.

A myeloperoxidase promoter polymorphism is independently associated with mortality in patients with impaired left ventricular function

Circulating levels of myeloperoxidase (MPO) predict adverse outcome in patients with impaired left ventricular (LV) function. The MPO -463 G/A promoter polymorphism (rs 2333227) regulates MPO transcription, with the G allele being linked to increased protein expression. The aim of this study was to assess the prognostic information derived from the -463 G/A MPO polymorphism on outcomes of patients with impaired LV function. The -463 G/A promoter MPO genotype as well as MPO plasma levels were determined in 116 patients with impaired LV function. Patients were prospectively followed for a median of 1050 days. The GG genotype was associated with a decrease in overall survival (chi(2) 5.80; p=0.016). This association remained after multivariate adjustment for plasma levels of NT-proBNP, creatinine, hsCRP, and MPO; leukocyte count; and LV function (hazard ratio 3.16 (95% CI 1.17-8.53), p=0.024) and for classical cardiovascular risk factors (hazard ratio 2.88 (95% CI 1.13-7.33), p=0.026). Interestingly, we observed no association of the MPO polymorphism with total MPO protein concentration or MPO activity in plasma. The -463 G/A MPO polymorphism is linked to adverse clinical outcome of patients with impaired LV function. Further studies are needed to elucidate the value of this polymorphism for risk stratification.

The effect of different molecular weight hyaluronan on macrophage physiology

Hyaluronan, a linear glycosaminoglycan, is an abundant component of extracellular matrix. In its native form, the high-molar-mass hyaluronan polymers have an array of structural and regulatory, mainly anti-inflammatory and anti-angiogenic, functions. In contradiction, the biological effects of fragmented low molecular weight hyaluronan are suggested to be pro-angiogenic and pro-inflammatory. The effects of highly purified pharmacological grade hyaluronan of defined molecular weights 11, 52, 87, 250 and 970 kilodaltons were tested on mouse macrophage cell lines RAW 264.7 and MHS. The surface expression of CD44 and Toll-like receptor 2, surface receptors for hyaluronan, was determined by flow cytometry. Activation of macrophages was determined based on nitric oxide and tumour necrosis factor alpha production, inducible nitric oxide synthase expression, and the activation of the nuclear factor kappa B transcriptional factor. Both macrophage cell lines expressed CD44 and Toll-like receptor 2, which were significantly increased by the pre-treatment of macrophages with bacterial lipopolysaccharide. Hyaluronan of any molecular weight did not activate production of nitric oxide or tumour necrosis factor alpha in any mouse macrophage cell lines. Correspondingly, hyaluronan of any tested molecular weight did not stimulate nuclear factor kappa B activation. Similarly, hyaluronan of any molecular weight neither exerted stimulatory nor inhibitory effects on macrophages pre-treated by lipopolysaccharide. Interestingly, the data does not support the current view of low molecular weight hyaluronan as a pro-inflammatory mediator for macrophages. Further studies are necessary to clarify the effects of different molecular weight hyaluronan on phagocytes.

The comparison of impedance-based method of cell proliferation monitoring with commonly used metabolic-based techniques

Determination of cell numbers is a crucial step in studies focused on cytokinetics and cell toxicity. The impedance-based analysis employing electronic sensor array system xCELLigence System allowing label-free dynamic monitoring of relative viable adherent cell amounts was compared with the most utilized methods for relative quantification of viable cell numbers based on a determination of cellular metabolism. In this study, colorimetric assay based on reduction of tetrazolium salt (MTT) by mitochondrial enzymes and chemiluminiscent assay based on intracellular adenosine triphosphate (ATP) determination were compared with the impedance-based system. Cell morphology was compared by microscopic evaluation. Normal human epidermal keratinocytes (NHEK) and normal human dermal fibroblasts (NHDF), together with 3T3 mouse fibroblast and HaCaT keratinocyte cell lines were employed. The progress of cell growth curves obtained by different methods during 72 hours reflected cell type and cell seeding densities. The impedance-based method was found to be applicable for the determination of the cell proliferation of 3T3 fibroblasts, HaCaT and NHDF, since the comparison of this method with ATP and MTT determinations showed a comparable results. In contrast, the proliferation of NHEK measured by the impedance-based method did not correlate with other methodological approaches. This could be accounted to the specific morphological appearance of these cells. The study shows the impedance-based detection of viable adherent cells is a valuable approach for cytokinetics and pharmacological studies. However, the specific morphological characteristics of cell lines have to be considered employing this method for determination of cell proliferation without using other reference methods.