Different mechanisms in the regulation of NF-κB and kinase cascades in inflammation

PhD Theses

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Abbreviations

General abbreviations

3-AB, 3-aminobenzamide; ***AP1***, activator protein 1; ***COX***, cyclooxygenase; ***ERK1/2***, extracellular signal-regulated kinase ½; ***FA***, ferulaldehyde; ***FCA***, ferulic acid; ***HMG,*** high-mobility group; ***4HQN,*** 4-hidroxyquinazoline; ***IFNγ***, interferon γ; ***IL***, interleukin; ***iNOS***, inducible NO-synthase; ***JNK***, c-Jun N-terminal kinase; ***LPS***, lipopolysaccharide; ***MAPK***, mitogen activated protein kinase; ***MD-2***, myeloid differentiation-2; ***MKK***, MAPK kinase***;*** ***MRI,*** magnetic resonance imaging; ***NF-κB***, nuclear factor-kappa B; ***PAMP***, pathogen-associated molecular pattern; ***PARP,*** poly-(ADP-ribose) polymerase; ***PI3***, phosphatidylinositol 3; ***p90RSK,*** protein of 90 kDa from the ribosomal subunit S6 kinase; ***PRR***, pathogen recognition receptor; ***PSS***, physiological saline solution; ***RNS***, reactive nitrogen species; ***ROS***, reactive oxygen species; ***TLR***, toll-like receptor; ***TNFα***, tumor necrosis factor α; ***TRAF-6***, TNF-receptor associated factor-6.

Abbreviations of treatment groups

CTRL, vehicle treated group; ***4HQN,*** treated with 4HQN alone; ***FA***, treated with FA alone; ***LPS***, treated with LPS alone; ***LPS + 4HQN***, treated with LPS and 4HQN; ***LPS + FA***, treated with LPS and FA

Introduction

Sepsis:

The word sepsis is derived from the Greek term *sapios,* for rotten or "to make putrid". Sepsis is a systemic host response against microorganisms invading in previously sterile tissues. It is a syndrome related to severe infections and is characterized by end-organ dysfunctions away from the primary site of the infection. Despite decades of efforts and significant advance in extensive research, sepsis remains the most common cause of death with a current estimate of at least 750,000 cases per year and 215,000 deaths annually, in the intensive care units, just in the USA. Sepsis is the third leading cause of death in developed societies equaling the cases of fatal acute myocardial infarction. Although there are many

effective antibiotics available, sepsis still remains a major cause of death, in part because antibiotics are able to eliminate bacteria but cannot control systemic inflammation.

LPS signaling:

The innate immune response is the first line of defense against infectious agents and is devoted to recognize highly conserved pathogen motifs the pathogen associated molecular patterns (PAMPs). Macrophage activation by lipopolisaccharide (LPS) a component of the Gram-negative cell wall has been extensively studied in an attempt to define the mechanisms that underlie innate immunity against bacterial pathogens. Binding of LPS to the CD14 and TLR4/MD2 complex transduces the signal via many different adapter proteins that trigger the signaling cascade. TLR4 uses the myeloid differentiation primary-response gene 88 adapter like protein (MAL) as a bridging adaptor to recruit the myeloid differentiation primary-response gene 88 (MyD88) that activates signaling pathways like mitogen activated protein kinase (MAPK) pathways via TNF-receptor associated factor 6 (TRAF6) and PI3K/Akt pathway. The activated pathways such as, MAPK pathways including extracellular signal-regulated kinase (p42/44 MAPK or ERK1/2), p38 kinase and c-Jun N-terminal kinase (JNK), as well as the phosphatidylinositol 3 kinase (PI3K)/Akt are capable of modulating functional responses through phosphorylation of other kinases and some important transcription factors, like nuclear factor-kappa B (NF-κB).

Transcription factors, NF-κB:

MAPKs and Akt can mediate the activation of nuclear transcription factors, such as activator protein 1 (AP-1) or the activation and nuclear translocation of cytoplasmic transcription factors such as NF-κB, which activate the expression of numerous genes.

This important inflammation related transcription factor the NF-κB is responsible for the gene expression of cytokines, chemokines, growth factors, cell adhesion molecules and some acute phase proteins, which are strongly involved in the LPS-induced inflammatory processes. NF-κB is ubiquitous in almost all animal cell types and mediates cellular responses against stimuli, such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens. Depending on its actual role, NF-κB has been linked to cancer, inflammatory and autoimmune diseases, viral infection and to improper immune development. Binding of specific molecules on cell-surface receptors, such as TNFR or TLR-4 activates NF-κB and leads to rapid changes in gene expression, which are chronically active in many inflammatory diseases, such as inflammatory bowel disease, arthritis, asthma and

sepsis, septic shock, which involve oxidative stress in their pathomechanism. Therefore, many natural products including anti-oxidants with anti-cancer and anti-inflammatory property have also been shown to inhibit NF-κB. It seems there is a connection between inflammation and cancer and the possible link can be NF-κB. These findings emphasize the value of drugs and natural products including polyphenols that regulate the activity of NF-κB.

Inflammatory cytokines and oxidative stress:

NF-κB plays a major role in inducing inflammatory processes during infection caused by Gram-negative bacteria. Endothelial and epithelial cells, as well as neutrophils, macrophages and lymphocytes produce powerful pro-inflammatory mediators via activation of the aforementioned transcription factors, especially tumor necrosis factor α (TNFα), interleukin (IL)-6, IL-1 and IL-8, E-selectin, inter-cellular adhesion molecule-1 (ICAM-1 also known as CD54), vascular cell adhesion molecule-1 (VCAM-1 also known as CD106), tissue factor (TF) and high-mobility group box-1 (HMGB-1) among many other inflammatory molecules playing important roles in the induction of sepsis. These cytokines trigger a beneficial inflammatory response that promotes local coagulation to confine tissue damage. However, the excessive production of these proinflammatory cytokines can be even more dangerous than the original stimulus, overcoming the normal regulation of the immune response and producing pathological inflammatory disorders.

Beside the inflammatory cytokines, the pathomechanism of septic shock involves oxidative stress via expression of the most important free radical producing enzymes such as cyclooxygenase-2 (COX-2) that produces superoxide ($O_2^{\bullet -}$), a reactive oxygen species (ROS) or inducible NO synthase (iNOS) that generate nitric-oxide, a reactive nitrogen species (RNS). Oxidative stress has been implicated in playing a crucial role in the pathogenesis of a number of diseases including neurodegenerative disorders such as Alzheimer's disease, sepsis and septic shock. Oxygen or nitrogen containing radicals are highly reactive and may cause cellular dysfunctions by modifying and inactivating proteins, lipids, DNA and RNA. Reactive oxygen species have been implicated in the regulation of the most important inflammation related transcription factor the NF-κB, which plays an important role in the development of septic shock.

Many antioxidants are shown to downregulate the NF-κB dependent inflammatory genes, like iNOS, TNF-α, IL-1β, and COX-2 by scavenging superoxide and peroxide in the LPS-stimulated macrophages. Furthermore, ROS and RNS can induce DNA-breaks in the

affected cells and thereby activate the nuclear enzyme poly-(ADP-ribose) polymerase (PARP), which can dramatically contribute to the tissue damaging processes of sepsis.

Pharmacological inhibition of PARP:

After publishing the first results, which proved the crucial role of PARP-1 in many disorders, the needs to develop and to test potent PARP-1 inhibitors were strongly increased. Studies with the traditional PARP inhibitor 3-aminobenzamid (3-AB), suggested that PARP activity has no or partial role in the mechanisms of septic shock. However, 3-AB is considered to be a poor inhibitor of PARP and has pronounced toxicity *in vivo*. On the other hand novel, potent PARP inhibitors were found to protect against LPS-induced tissue damage.

Polyphenols, ferulic acid and ferulaldehyde

Recently, a number of natural products or ingredients of traditional medicines and healthy foods such as resveratrol, curcumin and proanthocyanidins were extensively investigated, even subjected to clinical trials as anti-inflammatory agents. Since solubility of these compounds is limited, it is questionable whether their bioavailability could account for their pharmacological effect. Furthermore, recent publications show that polyphenols in healthy foods or drinks such as chocolate, red wine or beer are readily metabolized to phenolic acids and aldehydes by the microflora of the intestines, raising the possibility that these metabolites, rather than the original natural products or food ingredients, are responsible for their anti-inflammatory properties. Ferulic acid (FCA) and ferulaldehyde (FA) are potential end-products of dietary polyphenol degradation since they were found at a high concentration in human urine after red wine and chocolate consumption. Furthermore, FCA was reported to stay in the blood longer than other antioxidants such as vitamin C and have higher bioavailability than that of other dietary flavonoids and monophenolics studied so far.

FCA and FA are produced by the Shikimate pathway in plants and is a product of the phenylalanine and tyrosine metabolism. Because of its molecular structure, ferulic acid (4-hydroxy-3-methoxy cinnamic acid) belongs to the group of hydroxycinnamic acids. Ferulic acid, similarly to other hydroxycinnamic acids, is reported to be a potent antioxidant. The antioxidant potential of FCA can usually be attributed to its structural characteristics. The methoxy and hydroxyl groups terminates the free radical chain reactions as electron donating groups, and the C-C double bond can provide attack sites for free radicals.

In part, because of its strong antioxidant activity, FCA exhibits a wide range of therapeutic effects against various diseases such as cancer, diabetes, cardiovascular and

neurodegenerative disorders. Furthermore, it has been reported that ferulic acid or related ester derivatives decrease the levels of some inflammatory mediators such as prostaglandin E2, TNFα and iNOS expression and function in LPS stimulated cells. Hydrophobic ester derivatives of FCA were shown to have enhanced inhibitory activity on iNOS protein expression in LPS+IFNγ activated RAW 264.7 macrophages. It was reported that feruloyl-myoinositols, the derivatives of ferulic acid, suppressed cyclooxygenase-2 promoter activity in human colon cancer DLD-1 cells. It was also reported that FCA dose dependently inhibited the production of murine macrophages inflammatory protein-2 (MIP-2), a member of chemokine superfamily, in LPS-stimulated RAW 264.7 cells. These evidences strongly suggest that ferulic acid, as a potent antioxidant, has anti-inflammatory effect.

Its reduced form, ferulaldehyde (FA) possesses the same structural characteristic and main molecular motifs as ferulic acid up to the aldehyde group. Because of the structural similarity, FA is thought to have very similar or maybe better biological activity as ferulic acid, because of the reactive aldehyde group, which can potentially be oxidized to carboxylic group. In the literature there are numbers of reports explaining the effect of ferulic acid under numerous experimental conditions, but the effect of ferulaldehyde remains still unclear. In a few papers, published about FA, it was reported to inhibit LPS-induced iNOS expression and NO synthesis in murine macrophage-like RAW 264.7 cells and to have a good antioxidant activity in about the same degree as ferulic acid.

Aims of study

1. Although previously we provided evidence for the anti-inflammatory effect of PARP inhibition in a mouse model of septic shock and demonstrated significant involvement of the PI3K/Akt pathway in this effect, the exact signaling mechanisms still remained elusive. Therefore, our first aim was to identify other pathways implicated in the LPS-induced signaling mechanisms such as MAPK pathways, which can potentially be affected in the anti-inflammatory effect of PARP inhibition.

2. Based on *in vitro* evidences, some recent publications raised the possibility that microbial degradation products of polyphenols rather than the polyphenols themselves are

responsible for the anti-inflammatory and antioxidant effects of polyphenol containing healthy food, drink or traditional medicines. To provide *in vivo* experimental basis for this theory, our second aim was to investigate the effect of FA, an antioxidant and microbial metabolite of several polyphenols, in a mouse model of septic shock as well as in primary hepatocytes activated by LPS and IFNγ. Also, we intended to identify signaling mechanisms, transcription factors and inflammatory cytokines involved in the anti-inflammatory effect of FA.

3. PARP activation and oxidative stress can be both responsible for the activation and nuclear translocation of the most important inflammatory transcription factor the NF-κB. Therefore, we compared the effect of 4HQN, a PARP inhibitor with negligible antioxidant properties, and FA, a potent antioxidant with no effect on PARP, on regulation of LPS-induced inflammatory processes focusing mainly on the signaling mechanisms leading to NF-κB activation.

4. In addition many reports provide evidence about the altered sensitivity of differential mouse strains in response to many harmful stimuli, like LPS, which can be widely confirmed by our findings. But the molecular basis of this phenomenon has not been properly clarified. Accordingly, our last aim was to find molecular mechanisms potentially causing the observed different immunresponse, by the comparison of the signaling mechanisms induced by LPS, in our experimental animals derived from different strains.

Materials and methods

Animals

BALB/c and C57BL/6 mice were purchased from Charles River Hungary Breeding LTD. Animals were kept under standardized conditions; tap water and mouse chow were (CRLT/N, Szindbad Kft, Hungary) provided *ad libitum* during the whole experimental procedure. Animals received human care according to the Guide for the Care and Use of Laboratory Animals published by the US NIH, and the experiment was approved by the Animal Research Review Committee of the University of Pecs, Medical School. Animals were treated in compliance with the approved institutional animal care guidelines.

Materials

LPS from *Escherichia coli* 0127:B8 and 4-hydroxyquinazoline were purchased from Sigma/Aldrich Corporation (Saint Louis, MO or Budapest, Hungary); Primary antibodies, anti-phospho-p44/42 MAP kinase (Thr202/Tyr204), anti-phospho-Akt (Ser473, Thr308), anti-phospho-GSK-3β (Ser9), anti-phospho-SAPK/JNK (Thr183/Tyr185), anti-phospho-p90RSK (Thr359/Ser363) were from Cell Signalling Technology (Waltham, MA), anti-phospho-p38-MAPK (Thr180/Tyr182) was from Sigma, anti-COX-2 was from Santa Cruz Biotechnology (Santa Cruz, CA) and anti-HMG-1 was from Becton Dickinson (San Diego, CA). Ferulaldehyde was a generous gift of Prof. Kalman Hideg (Department of Organic and Pharmacological Chemistry, Faculty of Medicine, University of Pecs, Hungary).

Cell culture

Primary hepatocytes of C57BL/6 mice of 21-24 g body mass were isolated according to Le Cam A. (*Le Cam, 1993*) with slight modifications. Briefly, livers were perfused *in situ* with 50 mL of physiological saline solution (PSS) containing 6000 U/L heparin and 0.66 mmol/L EGTA followed by 50 mL of PSS then 35 mL of PSS containing 0.7 g/L collagenase H (Roche) and 10 mmol/L $CaCl_2$ at 37°C. Hepatocytes were seeded to 24- or 96-well plates coated with rat tail collagen type I (Sigma-Aldrich Ltd.) in DMEM containing 1% MEM non-essential amino acid solution, 0.05% insulin, 0.1% penicillin-streptomycin, 10% fetal calf serum and 0.1% dexamethasone.

Sepsis model

To induce murine endotoxic shock, BALB/c mice were injected intraperitoneally (i.p.) with LPS at a dose of 20 mg/kg in a volume of 250 µl. 4HQN (100 mg/kg) was administered i.p. in a volume of 250 µl three times a day proceeding the day of LPS-treatment, or as a single dose 1 or 6 hours following the LPS injection. Control mice received the same volume of sterile saline solution instead of the PARP-1 inhibitor.

To induce murine endotoxic shock in the C57BL/6 mice, animals were injected i.p. with a single dose of LPS (20 mg/kg assigned as low or 40 mg/kg assigned as high dose). FA (6 mg/kg) was administered i.p. in every 12 h, the first injection was given one hour before the LPS treatment. Mice treated with FA alone received 6 mg/kg FA, and control mice received the same volume of PSS instead of FA. The mice were monitored for clinical signs of endotoxemia and lethality every hour for 84 h, after which time they were monitored three times a day for 1 wk. No late deaths were observed in any of the experimental groups.

Western blot analysis

For Western blot analysis, groups of 4 BALB/c mice were pre-treated or not with 100 mg/kg 4HQN three times a day preceding the day of LPS-challenge (20 mg/kg). Liver, lung and spleen were removed from the animals 6 hours after the LPS treatment, were frozen in liquid N_2, and were processed exactly as described previously (*Veres et al., 2003*). Protein load was 35 µg/lane.

C57BL/6 mice were pre-treated with 6 mg/kg FA one h prior to LPS challenge (20 or 40 mg/kg). Livers were removed from the mice 1.5 h after the LPS treatment, were frozen in liquid N_2, and were processed exactly as described above. We applied the primary antibodies at $4^{\circ}C$ overnight at a dilution of 1:1000. The secondary antibodies were horseradish peroxidase-conjugated rabbit IgG. Peroxidase labeling was visualized with the SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Rockford, IL).

TNFα, IL-1β, IL-6, IL-10 determination

Mice were treated exactly as for Western blot analysis. Blood samples were taken 1.5 and 3 h after LPS administration, and were allowed to clot for 0.5 hour at room temperature before centrifuging for 20 minutes at 2000 x g. The serum was removed and assayed immediately. Selection of these time points was based on the published activation kinetics of the given cytokine. In these experiments, we used FA in 6 mg/kg dose injected 1 h before the LPS, which was applied in 20 mg/kg (low) and 40 mg/kg (high) doses. Serum TNFα, IL-1β, IL-6 and IL-10 concentrations were determined with the Quantikine M TNFα immunoassay kit (R&D Systems) and with IL-1β, IL-6, IL-10 ready-set-go kits (eBioscience). The ELISA-kits were used in accordance with the protocol of the manufacturer.

Determination of NF-κB and AP-1/c-Fos activation

Mice were treated exactly as for Western blot analysis. For nucleus isolation, liver, spleen and lung were removed 1.5 hours after the LPS-treatment, and were homogenized immediately according to the procedure described previously (Veres et al, 2003). Protein concentrations in nuclear extracts were determined using a bicinchoninic acid assay with bovine serum albumin (BSA) as standard (Sigma). To monitor NF-κB and AP-1/c-Fos activation in tissues, we used Trans-AMTM Transcription Factor Assay Kits (Active Motif, Rixensart, Belgium. The ELISA-kit was used in accordance with the protocol of the manufacturer.

MRI analysis

Mice were treated exactly as for Western blot analysis. Six hours after LPS treatment, the animals were anaesthetized with urethane (1.7 g/kg administered i.p.) and were placed into an epoxy resin animal holder tube. For the visualization of inflammatory response we performed T2-weighted MR-Images. *(Details about our NMR-spectrometer you can see in the dissertation!)*

Determination of ROS production

Culturing medium was replaced with a fresh one without dexamethasone, and the primary hepatocytes were incubated in the presence of 5 mg/L LPS + 50 μg/L interferon-γ (IFNγ) alone, or together with FA (1-100 μmol/L) for 24 h. Then 2,4-dichlorodihydrofluorescein-diacetate (C400, Invitrogen) at a final concentration of 2 mg/L was added to the medium for an additional two hours. Fluorescence was measured at 485 nm excitation and 555 nm emission wavelengths by using a Fluostar Optima (BMG Labtechnologies, Heidelberg, Germany) fluorescent microplate reader.

Measurement of nitrite concentration

Culturing medium was replaced with a fresh one without dexamethasone, and the primary hepatocytes were incubated in the presence of 5 mg/L LPS + 50 mg/L IFNγ alone, or together with FA (1-100 μmol/L) for 24 h. Then NO_2^- production was measured by adding to 50 μL culture supernatant equal volume of Griess-reagent (1% sulphanilamide, 0.1% naphthylethylenediamide in 5% phosphoric acid) and measuring light absorption at 550 nm by means of an Anthos 2010 (Rosys, Wiena, Austria) microplate reader.

Measurement of free radical scavenging activity

Oxidation of the redox dye dihydrorhodamine123 was induced by 10 μmol/L H_2O_2 and 60 μmol/L EDTA Fe^{2+} salt in the presence and absence of 5 to 100 μmol/L of FA or resveratrol. Fluorescent intensity of the oxidized dye was measured at 494 nm excitation and 517 nm emission wavelengths by using a LS50B spectrofluorimeter (Perkin-Elmer Ltd, Budapest, Hungary).

Statistical analysis

When pertinent, data were presented as means ± S.E.M. For multiple comparisons of groups, ANOVA was used. In some experimental models, because of the different doses (20 mg/kg and 40 mg/kg) of LPS, data were analyzed using one-way or two-way ANOVA followed by Bonferroni's correction. When F-test indicated unequal variances, Kruskal-Wallis test was used. For survival experiments, Mantel-Cox's logrank test was used. Differences were considered statistically significant at $P<0.05$.

Conclusion

In our experiments we investigated the effect of 4HQN, a PARP inhibitor with negligible antioxidant properties, and FA, a potent antioxidant with no effect on PARP, on LPS-induced inflammatory processes in BALB/c and C57BL/6 mice, respectively.

1. Our experiments underlined that PARP activation is critically involved in the pathogenesis of sepsis and inhibition of this nuclear enzyme is able to reduce the activation of the most important sepsis related transcription factor, the NF-κB via modulating signal transduction pathways such as PI3K/Akt as well as ERK and p38-MAPK in a tissue specific manner in mice.

2. We found that FA, a microbial metabolite of several polyphenols has anti-inflammatory effect via modulation of kinase cascades such as JNK and Akt, the transcription factor NF-κB and some inflammatory cytokines such as TNFα, IL-1β, IL-6, IL-10 in mice. Furthermore, elimination of ROS and RNS contributed to this anti-inflammatory effect as we demonstrated in primary hepatocytes and in a cell free system. Accordingly, phenolic acids and aldehydes, as potential microbial degradation products of several polyphenols, with higher bioavailability than their parent molecules can at least contribute to the anti-inflammatory effect of their parent polyphenols.

3. According to our data, FA, an antioxidant and 4HQN, a PARP inhibitor both inhibited LPS-induced inflammatory processes, but via a completely different mechanism.

4HQN inhibited PARP, the transcriptional co-activator of NF-κB, and enhanced LPS-induced overactivation of Akt thereby increased the activity of this cytoprotective pathway. On the other hand, FA prevented nuclear translocation of NF-κB by scavenging ROS and RNS as well as by inhibiting the pro-inflammatory JNK signaling.

4. We found that LPS did induce Akt activation in C57BL/6 while did not at all affected it in BALB/c mice. Also, JNK was activated by LPS in C57BL/6 while unchanged in BALB/c mice. Considering the importance of these kinase signaling pathways in the inflammatory process, these findings can explain the altered sensitivity toward LPS of these mouse strains.

These results about the effect of 4HQN and FA in LPS-induced endotoxic shock can confirm the overall picture about the role of NF-κB in inflammation and gives another insight into the complex world of signaling mechanism leading to the modulation of this transcription factor.

Publications

- Publications in the topic -

Articles:

Radnai B, Tucsek Z, Bognar Z, Antus C, Mark L, Berente Z, Gallyas F Jr, Sumegi B, Veres B.: Ferulaldehyde, a water-soluble degradation product of polyphenols, inhibits the lipopolysaccharide-induced inflammatory response in mice. J Nutr. 2009 Feb;139(2):291-7. Epub 2008 Dec 23.

Veres B, **Radnai B**, Gallyas Jr F, Várbíró G, Berente Z, Ösz E, Sümegi B.: Effect of poly(ADP) ribose polimerase-1 inhibitor - 4-hydroxyquinaziline - on LPS-induced inflammation: modulation of kinase cascades and activation of transcription factors. J. Pharmacol. Exp. Ther. 2004 Jul;310(1):247-55. Epub 2004 Mar 03.

Oral presentations:

Radnai Balázs: A ferulaldehid, egy polifenol degradációs termék hatása LPS indukálta endotoxikus sokkra egérben. Biológus Doktoranduszok Konferenciája. A Pécsi Akadémiai Bizottság Biológiai Tudományok Szakbizottságának rendezvénye, 2009. november 12-13.

Zsuzsanna Tucsek, **Balazs Radnai**, Tamas Dolowschiak, Janos Priber, Csenge Antus and Balazs Veres: Ferulaldehyde, a degradation product of polyphenols, inhibits the lipopolysaccharide-induced inflammatory response in mice and in RAW 264.7 cells. 13th Congress of the European Shock Society, Lisbon, Portugal, 24th-26th September 2009

Radnai Balázs, Tucsek Zsuzsanna, Hocsák Enikő, Vető Sára, Németh Viktória, Bognár Eszter, Grász Dénes, Berente Zoltán, ifj.Gallyas Ferenc, Sümegi Balázs: A ferulaldehid hatása az LPS indukálta endotoxikus sokkra egerekben. A Magyar Biokémiai Egyesület 2006. évi Vándorgyűlése, Pécs, 2006. augusztus 30- szeptember2.

Posters:

Tucsek Zsuzsanna, **Radnai Balázs**, Veres Balázs, Dolowschiák Tamás, Woth Gábor László, Pribér János, Schoenberg Markus és ifj.Gallyas Ferenc: A ferulaldehid hatása LPS-indukálta gyulladásos folyamatokra egérben. 37. Membrán-Transzport Konferencia, Sümeg, 2007. május 22-25.

F. Gallyas Jr., B. Veres, **B. Radnai**, E. Hocsak and B. Sumegi: Significant role of cytoprotective kinase signaling mechanisms in the protective effect of PARP inhibitors in a murine septic shock model. Budapest, Hungary, 30th FEBS Congress and 9th IUBMB Conference, 2-7 July, 2005

Veres B., **Radnai B.,** Gallyas F. Jr, Varbiro G., Berente Z., Osz E., Sumegi B.: Effect of poly(ADP-ribose) polymerase-1 inhibitor – 4-hydroxyquinazoline – on LPS-induced inflammation: modulation of kinase cascades and activation of transcription factors. Debrecen, Hungary, FEBS Advanced Course: Poly-ADP-ribosylation in health and disease. 2003. August 27-30.

Veres B., Tapodi A., **Radnai B**., Varbiro G., Sumegi B.: A 4-hidroxykinazolin hatása ay LPS-indukálta szeptikus sokkra. Sümeg, XXXIII. Membrán-Transzport Konferencia, 2003. Május 21-24.

- Further publications -

Chapter in a book:

Sumegi B., Kovacs K., Veres B., **Radnai B**., Varbiro G., Bognar Z., Toth A., Gallyas F. Jr.: Oxidative Stress and the Endoplasmatic Reticulum. In: Benedetti A., Banhegyi G., Burchell A. (Eds). Endoplasmatic reticulum: A metabolic compartment. IOS Press, pp.121-130. (2005)

Articles:

Bognar Z, Kalai T, Palfi A, Hanto K, Bognar B, Mark L, Szabo Z, Tapodi A, **Radnai B**, Sarszegi Z, Szanto A, Gallyas F Jr, Hideg K, Sumegi B, Varbiro G.: A novel SOD-mimetic

permeability transition inhibitor agent protects ischemic heart by inhibiting both apoptotic and necrotic cell death. Free Radic. Biol. Med. 2006 Sep 1;41(5):835-48. Epub 2006 Jun 15.

Oral presentations:

Sümegi B., Kovács K., Tapodi A., Hantó K., **Radnai B.**, ifj. Gallyas F., Hocsák E., Hideg K.: Az ADP-riboziláció szerepe a PI3-kináz, Akt és MAPK jelátviteli útvonalak szabályozásában. Eger, XIII. Sejt és Fejlődésbiológiai Napok, 2005. április 10-12.

Veres B., **Radnai B**., Gallyas F. Jr, Varbiro G., Berente Z., Osz E., Sumegi B.: Effect of poly(ADP-ribose) polymerase-1 inhibitor – 4-hydroxyquinazoline – on LPS-induced inflammation: modulation of kinase cascades and activation of transcription factors. Pecs, IV. International Symposium on Myocardial Cytoprotection, 2003. szeptember 25-27.

Posters:

Zsuzsanna Tucsek, **Balazs Radnai**, Tamas Dolowschiak, Janos Priber, Csenge Antus and Balazs Veres: Ferulaldehyde, a degradation product of polyphenols, inhibits the lipopolysaccharide-induced inflammatory response in mice and in RAW 264.7 cells. 13th Congress of the European Shock Society, Lisbon, Portugal, 24th-26th September 2009

Zsuzsanna Tucsek, Tamas Dolowschiak**, Balazs Radnai**, Balazs Veres, Ferenc Gallyas Jr. and Balazs Sumegi: Effect of ferulaldehyde on inflammatory response in RAW macrophages and mice. 8th World Congress on Inflammation, Copenhagen, 16-20 June 2007.

Zsuzsanna Tucsek, **Balazs Radnai**, Zoltan Szabo, Tamas Dolowschiak, Eniko Hocsak, Aliz Szabo, Jr. Ferenc Gallyas, Tamas Lorand, and Balazs Sumegi: The effect of the IK11, an E - 4 - arylidene-3-isochromanone on the Hep G2 human hepatocellular carcinoma cell line. Semmelweis Symposium – Nitric Oxide and Nitrosative stress in the Cardiovascular System, Budapest, 2006. okt. 29-31.

Balazs Radnai, Zsuzsanna Tucsek, Balazs Veres, Katalin Hanto, Peter Jakus, Balazs Debreceni, Zita Bognar, Eniko Hocsak, Denes Grasz, Ferenc Gallyas Jr. and Balazs Sumegi: Effect of a PARP inhibitor, HO-3089 on the gene expression profiles of LPS stimulated RAW 264,7 murine macrophages. Semmelweis Symposium – Nitric Oxide and Nitrosative stress in the Cardiovascular System, Budapest, 2006. okt. 29-31.

Tucsek Zsuzsanna, **Radnai Balázs**, Szabó Zoltán, Dolowschiák Tamás, Hocsák Enikő, Szabó Alíz, Solti Izabella, Bognár Eszter, Vető Sára, ifj.Gallyas Ferenc, Loránd Tamás és Sümegi Balázs: A PJ34 protektív hatása az IK11 indukálta oxidatív stresszben HepG2 sejtvonalon. 36. Membrán-Transzport Konferencia, Sümeg, 2006. május 23-26.

Radnai Balázs, Tucsek Zsuzsanna, Szabó Zoltán, Dolowschiák Tamás, ifj.Gallyas Ferenc, Tamás Loránd és Sümegi Balázs Az IK11, egy E-4-arilidén-3-izokromanon hatása a Hep G2 humán hepatocelluláris karcinóma sejtvonalon. Magyar Szabadgyökkutató Társaság III. Konferenciája, Debrecen, 2005. okt.13-15.

F. Gallyas Jr., Zita Bognar, **Balazs Radnai**, Antal Tapodi, Katalin Hantó, Aliz Szabó, Viktor Soma Poór, Peter Jakus, Gabor Várbiró, Balazs Sumegi: Poly-(ADP-ribose) polymerase inhibitors influence the taxol induced cell death in cultured cells.Gateshead, United Kingdom, Poly-(ADP-ribose) polymerase: Bench to bedside, 5-7 October, 2005.

B. Radnai, B. Veres, K. Hantó, P. Jakus, G. Varbiro, Z. Bognar, A. Tapodi, S. Veto, and B. Sumegi: The effect of a novel PARP inhibitor HO-3089 on the LPS stimulated RAW264,7 murine macrophage cells. Budapest, Hungary, 30th FEBS Congress and 9th IUBMB Conference, 2-7 July, 2005

K. Hantó, **B. Radnai**, Z. Bognar, V. Nemeth, G. Varbiro, A. Tapodi, F. Gallyas Jr., B. Sumegi and K. Toth: NFκB dependent induction of COX-2 expression by amiodarone in liver cells. Budapest, Hungary, 30th FEBS Congress and 9th IUBMB Conference, 2-7 July, 2005

Z. Bognar, G. Varbiro, **B. Radnai**; A. Tapodi, K. Hanto, P.Jakus, F. Gallyas Jr and B. Sumegi: The effect of poly(ADP)ribose polymerase inhibitors on taxol induced cell death in cultured cells. Budapest, Hungary, 30th FEBS Congress and 9th IUBMB Conference, 2-7 July, 2005

Radnai B., Veres B., Hantó K., Jakus P., Tapodi A., Várbíró G., Bognár Z., Vető S. és Sümegi B.: Egy új PARP-gátló szer a HO-3089 hatása az LPS stimulálta egér makrofág sejtvonalon. Eger, XIII. Sejt és Fejlődésbiológiai Napok, 2005. április 10-12.

Radnai B., Veres B., Várbiró G., Tapodi A., Bognár Z.,Sümegi B.: A poli-(ADP-ribóz)-polimeráz gátlás hatása az endotoxin stimulálta egér makrofág sejtvonalon XXXIV. Membrán Transzport Konferencia, Sümeg, 2004. június 1-4.

Radnai B., Veres B., Várbiró G., Tapodi A., Bognár Z.,Sümegi B.**:** The role of 4-HQN - a PARP-1 inhibitor - on LPS stimulated murine macrophage cells. Dubrovnik, Croatia, FEBS Lecture Course on Cellular Signaling & 4th Dubrovnik Signaling Conference, May 21-27, 2004

Bognar Z., Varbiro G., Palfi A., Veres B., Tapodi A., **Radnai B.,** Sumegi B.: An amiodarone anlogue, HO3538 protects ischemic hearts by inhibiting the mitochondrial apoptotic pathway. Istanbul, Turkey. 4th European Workshop on Cell Death. 2004. May 11-16.

Varbiro G., Bognar Z., Veres B., Tapodi A., **Radnai B**., Sumegi B.: Phosphatidic acid induces permeability transition and the collapse of the membrane potential in isolated rat liver mitochondria. Istanbul, Turkey. 4th European Workshop on Cell Death. 2004. May 11-16.

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