Effect of a red wine compound on LPS-induced inflammatory processes *in vivo* and *in vitro*

PhD Theses

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2011.

Abbreviations

AP-1, activator protein-1, **CAT**, catalase, **COX-2**, cyclooxygenase-2, **DSP**, dual-specificity protein phosphatase, **ELAM**, endothelial leukocyte cell adhesion molecule, **ERK1/2**, extracellular signal-regulated kinase, **FA**, ferulaldehyde, **GSHPx**, glutathione peroxidase, **GSSGR**, glutathione reductase, **HMGB-1**, high-mobility group box-1 protein, **ICAM**, intercellular adhesion molecule, **IFN-γ**, interferon-γ, **IKK**, IκB kinase, **IL**, interleukin, **iNOS**, inducible NO-synthase, **JNK**, c-Jun N-terminal kinase, **LBP**, lipopolysaccharide binding protein, **LPS**, lipopolysaccharide, **M3K**, MKK kinase, **MAPK**, mitogen-activated protein kinase, **MKK**, MAPK kinase, **MKP**, MAP kinase phosphatase, **NF-κB**, nuclear transcription factor-kappa B, **NO**, nitric-oxide, **PAMP**, pathogen-associated molecular pattern, **PI-3K**, phosphoinositide-3 kinase, **PRR**, pathogen recognition receptor, **RNS**, reactive nitrogen species, **ROS**, reactive oxygen species, **SAPK**, stress-activated protein kinase, **SIRS**, systemic inflammatory response syndrome, **SOD**, superoxide dismutase, **TLR**, toll-like receptor, **TNF-α**, tumor necrosis factor-α, **VCAM**, vascular cell adhesion molecule

Introduction

Sepsis and septic shock

A tight regulation of the immune/inflammatory system is crucial for maintaining the balance between protective and tissue-damaging responses. Systemic inflammatory response syndrome (SIRS) and sepsis are characterized by a loss of control over inflammatory responses, which can be provoked by a variety of causative agents and severe clinical insults. Typical clinical symptoms are manifested during SIRS and sepsis, such as hypothermia/hyperthermia, tachycardia, tachypnea and leukocytopenia/leukocytosis. Sepsis is defined as ‘severe’ when these symptoms are associated with hypotension, hypoperfusion, and different multiple organ dysfunctions, such as oliguria, lactic acidosis, elevated liver enzymes or altered cerebral function. Severe sepsis is a less acute syndrome with a mortality rate of 30–70%, in contrast to septic shock, which is highly lethal - kills within 24–48 h after onset - and marked by hypotension despite fluid resuscitation. Despite of use of more potent and broader-spectrum antibiotics, immunosuppressive agents and invasive technology, sepsis

remained the most common cause of death in intensive care units and affects over 18 million people worldwide with an expected 1% increase of incidence per year.

LPS signaling

Aberrant innate immune response of lymphocytes, monocytes, and macrophages to infection has been implicated into triggering dysregulated hyper-inflammatory responses in sepsis. Pathogen recognition is one of the most basic and important properties the immune system, that is fulfilled by pathogen recognition receptors (PRRs) of immune cells. Genotypically encoded PRRs, for example the toll-like receptors (TLRs), recognize pathogen-associated molecular patterns (PAMPs) of potentially pathogenic microorganisms (e.g. lipopolysaccharide (LPS) of Gram-negative bacteria). In macrophages and monocytes, LPS binding to LPS binding protein (LBP) and then transferred to their extracellular receptor complex, CD14-MD2-TLR4 has been shown to initiate multiple intracellular signaling events, including the activation of IκB kinase (IKK)/ nuclear transcription factor (NF)-κB pathway; mitogen-activated protein kinases (MAPKs); and phosphoinositide-3 kinase (PI-3K)/Akt pathway. These signaling pathways in turn activate a variety of transcription factors that include NF-κB (p50/p65) and activator protein-1 (AP-1, c-Fos/c-Jun). The activation of these transcription factors ultimately leads to the synthesis and release of diverse mediators of inflammation, pro-inflammatory cytokines and chemokines (tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-12 (IL-12), high-mobility group box-1 protein (HMGB-1) etc.), as well as the expression of cyclooxygenase-2 (COX-2), inducible NO-synthase (iNOS) and the upregulation of cell adhesion molecules (intercellular adhesion molecules (ICAMs), endothelial leukocyte cell adhesion molecules (ELAMs, E-selectin), platelet endothelial cell adhesion molecule-1 (P-selectin) and vascular cell adhesion molecules (VCAMs)). Overexpression of COX-2 and iNOS can lead to the production of reactive oxygen species (ROS) and nitric-oxide (NO) respectively, as prime tools of antimicrobial function of activated macrophages. Secretion of pro-inflammatory cytokines, upregulation of cell adhesion molecules and production of ROS and NO lead to a continued stimulation of epithelial and endothelial cells that in turn might activate the NF-κB transcription factor. Thus, a positive autoregulatory loop might be established that can amplify the inflammatory response and finally leads to enhanced systemic inflammation, endothelial dysfunction and organ failure.

MAPK signaling, MKP-1

MAPK-signal transduction pathways are highly conserved cascades important in diverse aspects of the immune response. They form a family of protein kinases that include extracellular signal-regulated kinase (p42/44 MAPK or ERK1/2), p38 MAP kinase and stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK). MAPKs may be present in cytosolic, mitochondrial and nuclear compartments and are activated by upstream MAPK kinases (MKKs), which in turn are activated by MKK kinases (M3Ks). While activation of MAPK signal transduction cascades is critical for mounting an aggressive immune response to eliminate invading pathogens, deactivation of the signaling pathways restrains the potentially devastating actions of the immunological system on the host, thus preventing self destruction. These negative regulators modulate the strength and duration of the transduced signals. MAP kinase phosphatases (MKPs) have a central role in the restraint of innate immune response and the prevention of septic shock syndrome during pathogenic microbial infection. In mammalian cells, MKPs, also called dual-specificity (tyrosine and serine/threonine) protein phosphatases (DSPs) are the primary phosphatases responsible for dephosphorylation and deactivation of MAP kinases. To date, at least eleven MKPs have been identified in mammalian cells, with MKP-1 being the archetype which is a MAP kinase-selective protein phosphatase. MKP-1 is a critical negative regulator of macrophage signaling in response to inflammatory stimuli and through deactivation of MAKPs it can switch off the production of pro-inflammatory cytokines.

Transcription factors and cytokines

NF-κB is involved in the control of a variety of genes activated upon inflammation, and it plays a central role in the inflammatory response to infection. Binding of LPS to TLR triggers a cascade of cellular signals, culminating in the eventual activation of NF-κB and AP-1 transcription factors, which bind to a discrete nucleotide sequence in the upstream regions of genes that produce pro-inflammatory cytokines, chemokines and adhesion molecules such as TNF-α, IL-1, IL-6, IL-8, IL-12, ICAM-1, E-selectin, P-selectin, VCAM-1 and HMGB-1 thereby regulating their expression.

TNF-α and IL-1β play a pivotal role in the early phase of immune response against infections and in part in the organ dysfunction related to septic shock. Plasma levels of TNF-α and IL-1β peak at the early stage within minutes after infection, but after 3–4 h secretion stops and their levels become almost undetectable in the late stage (in severe sepsis). TNF-α amplifies inflammatory cascades in an autocrine and paracrine manner by activating

macrophages and monocytes to secrete other pro-inflammatory cytokines. IL-10 has an increased production in the late phase of sepsis is believed to contribute to 'immunosuppression'.

ROS production in inflammation

Beside the inflammatory processes, oxidative stress is also involved in the pathomechanism of sepsis and reactive oxygen and nitrogen species (ROS/RNS) are important mediators of cellular injury during endotoxemia. iNOS is expressed and continuously active during inflammation, where it is involved in host-defense against pathogens. iNOS generates NO which can be converted to its stable products, nitrite and nitrate. ROS are generated during normal cellular metabolism. The respiratory chain in mitochondria is the major source of oxygen radicals, but, there are other possible sources of ROS in inflammatory processes such as metabolic cascade of arachidonic acid (via COX-2), xanthine-oxidase and NADPH oxidase. Increased ROS and RNS cause peroxidation of membrane phospholipids, oxidation of proteins and DNA damage which processes lead to altering of membrane fluidity, loss of cellular integrity, decrease of energy levels in the cell and finally cell and tissue damages. Furthermore oxidative damage has been implicated in playing a crucial role in pathogenesis of a number of diseases including neurodegenerative disorders, such as Alzheimer's disease, cardiovascular alterations, sepsis and septic shock.

Aerobic organisms have developed an array of defense mechanisms against ROS damage, which represent a fine balance between ROS generation and antioxidant defense in cells. This system includes antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), and glutathione reductase (GSSGR), which protect by directly scavenging superoxide radicals and hydrogen peroxide, converting them to less reactive species. Non-enzymatic defense system includes compounds of intrinsic antioxidant properties, such as vitamins C and E, glutathione, and ß-carotene. A variety of antioxidants have been reported to potently decrease free radical production and suppress the activation of NF-κB both *in vivo* and *in vitro.* These results suggest that antioxidants which are effective against oxidative damages may be beneficial in preventing endotoxin-induced inflammatory processes.

Polyphenols

A wide variety of dietary plants including grains, berries, legumes, tea, beer, grape/wine, olive oil, chocolate/cocoa, coffee, walnuts, peanuts, spices etc. contain

polyphenols. As antioxidants, polyphenols are normally produced by plants for their antibiotic and antifungal features. Recently, a number of natural products or ingredients of traditional medicines and healthy foods such as resveratrol, curcumin, and catechins were extensively investigated and subjected to clinical trials as anti-inflammatory agents. Although the knowledge of absorption, bioavailability and metabolism of polyphenols is not entirely known, it appears that some polyphenols are bioactive and are absorbed in their native or modified form.

The active components of dietary phytochemicals (e.g. curcumin, resveratrol, capsaicin, catechins, vitamins, beta carotene and dietary fiber) are believed to suppress the inflammatory processes, moderate cell signaling pathways, proliferation, apoptosis, redox balance and most often appear to be protective against cancer, neurodegenerative disorders and cardiovascular diseases. Polyphenols can exert their anti-inflammatorical properties at multiple levels, through the modulation of MAPK, Akt and NF-κB signaling pathways, inhibition the production of inflammatory cytokines and chemokines, suppressing the activity of COX and iNOS and decreasing the production of ROS/RNS.

Because of the limited solubility of polyphenols, it is questionable whether their bioavailability could account for their pharmacological effect. Recent publications show that polyphenols in healthy foods 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. Ferulaldehyde (FA) is a water-soluble end-product of dietary polyphenol degradation, because it was found at a high concentration in human urine after red wine and chocolate consumption, and its oxidized form ferulic acid (4-hydroxy-3-methoxy cinnamic acid) 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.

Ferulic acid and FA are products of the phenylalanine and tyrosine metabolism, and they are produced by the shikimate pathway in plants. The antioxidant potential of ferulic acid can usually be attributed to its structural characteristic. Three distinctive motifs (3-methoxy and 4-hydroxy groups on the benzene ring, and the carboxylic acid group) of ferulic acid are responsible for its free radical scavenging capability. Partially, because of its antioxidant and anti-inflammatory activity, ferulic acid is considered as a potential therapeutic agent (together with other naturally phenolic compounds) against various diseases like cancer, diabetes, cardiovascular dysfunction, inflammatory diseases and neurodegenerative diseases. The

structural characteristic of ferulic acid and FA mainly resembles, the difference is one functional group. Due to this structural similarity and the presence of the reactive aldehyde group (which can be easily oxidized to carboxylic group), FA, is thought to have very similar or maybe better biological activity as ferulic acid.

Aims of the study

1. Using *in vitro* experimental systems, some recent publications raised the possibility that the anti-inflammatory properties of polyphenols in natural products, traditional medicines and healthy foods based on highly soluble metabolites produced by the microflora of the intestines rather than the polyphenols themselves. To provide *in vivo* experimental basis for this theory, our first aim was to investigate the anti-inflammatory features of ferulaldehyde, a natural end-product of polyphenol metabolism of intestinal microflora, in a murine LPS-induced septic shock model as well as in primary hepatocytes activated by LPS and interferon- γ (IFN-γ). Also, we intended to identify signaling mechanisms, transcription factors and inflammatory cytokines involved in the anti-inflammatory effects of FA.

2. Our *in vivo* data provide direct evidences that ferulaldehyde, a water-soluble end-product of dietary polyphenol degradation, exerted its beneficial anti-inflammatory effects during the early phase of inflammation. Macrophages represent the first defense line against bacterial infection and play a crucial role in early inflammatory response, therefore our second aim was to identify the effects of ferulaldehyde on the signaling mechanisms in an *in vitro* model, utilizing LPS-induced RAW 264.7 macrophage cells.

3. LPS treatment and oxidative stress trigger MAPK activation. MKP-1 was reported to dephosphorylate all three MAP kinases and it was found to be a critical negative regulator in the innate immune response to LPS. Based on these facts our third aim was to investigate the role of MKP-1 activation in regulation of early inflammatory response in RAW 264.7 macrophage cells.

4. Since *in vivo* and *in vitro* experimental systems have fundamental differences in several aspects we wanted to investigate and compare the signaling mechanisms in our models, especially focusing on regulation of MAPK pathways.

Results

Ferulaldehyde reduced LPS-induced mortality in mice

In our *in vivo* experiments we used FA at 6 mg/kg concentration and LPS in two different concentrations; in a 20 mg/kg (*sublethal or lower*) dose for determine the molecular mechanism of the drug and a 40 mg/kg (*lethal or higher*) dose for survival experiments. In our survival study the higher dose of LPS (40 mg/kg, i.p.) resulted in about 80% death of the animals within 36-48 h. C57BL/6 mice treated with FA 1 h before the LPS challenge (LPS + FA group) and repeated every 12 h had a longer survival time compared to those that received physiological saline solution. 36-48 h after the LPS challenge 70% of mice in LPS + FA group survived compared with 20% in the LPS only group. Although FA was not able to prevent eventual death (100 h after LPS treatment), it significantly increased the survival time of mice showing that it was able to positively influence the complex processes leading to LPS-induced oxidative stress and inflammatory response. This protective effect of FA was more pronounced on the early phase of LPS-mediated inflammatory processes of septic shock rather then on the late stage severe sepsis. FA treatment alone did not induce death or any obvious damage.

Ferulaldehyde inhibited LPS-induced inflammatory response in vivo

The in vivo response to LPS-induced endotoxic shock was detected by MR-Imaging techniques. T2-weighted images of control, LPS and LPS + FA-treated mice were taken 6 h after the LPS challenge. The lower abdominal region of LPS-treated mice showed marked increase of intensity (inflammation) especially in the lateral subcutaneous regions and the interintestinal cavities as well as around the kidneys. In mice treated with LPS + FA, the T2-weighted intensities were markedly lower, so the inflammatory response was significantly smaller. T2-weighted images of mice treated with FA alone were basically identical to images of untreated mice.

Ferulaldehyde attenuated LPS-induced TNF-α, IL-1β but not IL-6 production, and enhanced IL-10 generation in the sera of mice

In several previous reports, a direct link between TNF-α and IL-1β as well as IL-10 was established, however, contradictory results appeared about the role of IL-6 in the inflammatory response. In our LPS-induced endotoxic shock model even the lower LPS dose (20mg/kg), that caused only negligible death rate among the mice, induced significant induction of all the pro-inflammatory cytokines tested. We measured the concentration of various cytokines from sera of control, LPS-, LPS + FA- and FA-treated mice 1.5 or 3 h after the LPS challenge using enzyme-linked immunosorbent assay (ELISA)-based kits. Our finding that FA could not attenuate LPS-induced elevation of serum IL-6 level indicates that IL-6 was not involved among the anti-inflammatory mechanisms of FA in our murine septic shock model. However, FA attenuated the LPS-induced *in vivo* inflammatory response not only by attenuating TNF-α- and IL-1β pro-inflammatory cytokine production but also by enhancing anti-inflammatory IL-10 production.

Feruladehyde inhibited LPS+IFN-γ-induced NO_2^- and ROS production in primary hepatocytes

Eliminating or inhibiting the production of reactive oxygen and nitrogen species has been shown to attenuate inflammatory damages. However, the pathological changes that were previously observed in the liver of LPS-treated mice could be resulted from ROS and NO produced by the hepatocytes themselves. Therefore we determined FA's effect on LPS + IFN-γ-induced ROS and NO_2^-production in primary hepatocytes by measuring these in the culturing medium following a 24-h incubation using a fluorescent redoxi dye and a Griess reagent based assay, respectively. We used 5 mg/L LPS combined with 50 μg/L IFN-γ in order to induce full activation of the hepatocytes. During a 24-h incubation period, LPS + IFN-γ increased ROS and NO_2^- production was completely abolished by FA at the concentration of 50 μmol/L. Highest concentration of FA (100 μmol/L) decreased ROS and NO_2^- concentration below the control, or to the level of untreated control, respectively.

Ferulaldehyde inhibited LPS-induced ROS and NO_2^- production in RAW 264.7 macrophages

Macrophages represent the first defense line against pathogens and play a crucial role for the induction of the protective response to eliminate infectious agents producing pro-

inflammatory cytokines, reactive oxygen and nitrogen species. Therefore, we determined FA's effect on LPS-induced ROS and NO production in RAW macrophage cells by using a fluorescent redoxi dye and a Griess reagent based assay, respectively. We induced ROS and NO production by treating the cells with 100 ng/ml LPS for 24 h that was found not to have considerable cytotoxic effect under these conditions. Similarly to our finding in the model of primary hepatocytes, we found that the amount of both reactive oxygen and nitrogen species induced by LPS was reduced by FA in a concentration-dependent manner. Since at 50 µM concentration FA diminished LPS-induced ROS and nitrite accumulation in the cells to below and close to the control level, respectively; and this concentration was comparable to the one (6 mg/kg~33.6 µM) we used *in vivo*, we applied FA at a concentration of 50 µM in all *in vitro* experiments.

Ferulaldehyde protected the mitochondrial membrane potential in RAW 264.7 macrophages

Respiratory chain in mitochondria is one of the major sources of oxygen radicals. In turn, increased ROS leads to simultaneous collapse of mitochondrial membrane potential (ΔΨm) and a transient increased ROS generation by the electron transfer chain. We investigated ΔΨm using a cell-permeable voltage-sensitive fluorescent mitochondrial dye, JC-1 that emits green fluorescence when the mitochondria are depolarized, and red for normal ΔΨm when excited at 488 nm. Flow cytometry analysis revealed that 100 ng/ml LPS induced substantial mitochondrial depolarization at all time points (5, 10 30, 60 min) which was diminished by FA. Massive depolarization of mitochondria by LPS indicated by faint green fluorescence was also demonstrated by fluorescent microscopy performed 30 min after 100 ng/ml LPS challenge. ΔΨm was preserved by 50 µM FA as indicated by appearance of red fluorescence emitted by JC-1 aggregates in the mitochondria. Protection of mitochondria against LPS-induced rapid and massive membrane depolarization by FA indicate the importance of mitochondrial integrity in early inflammatory response.

Ferulaldehyde negatively regulated LPS-induced phosphorylation of JNK and Akt, and activation of NF-κB in liver

Signaling mechanisms leading to systemic tissue damages induced by binding of LPS to the CD14/TLR4/MD2 complexes involve activation of MAPKs, Akt pathway and AP-1 and NF-κB transcription factors. On the basis of these facts, we determined the effect of FA on MAPK, Akt and NF-κB activation from the livers of LPS-treated mice. In our

experiments we measured phosphorylation of JNK, ERK1/2, p38 MAPK and Akt from the liver of control, LPS-, LPS + FA- and FA-treated mice 1.5 h after the LPS challenge by Western-blotting. Phosphorylation and thereby activation of all the kinases studied, except p38 MAPK, were increased following LPS stimulation. FA significantly prevented this activation in the case of JNK and Akt, but failed to attenuate LPS-induced activation of ERK1/2. FA alone did not exert any effect on the phosphorylation of the kinases studied in our experimental model.

NF-κB activation and nuclear translocation was examined from the liver of control, LPS-, LPS + FA- and FA-treated mice 1.5 h after the LPS challenge by transcription factor assay. We found a nearly four-fold activation of NF-κB after LPS challenge as compared to the control liver. LPS-induced activation and nuclear translocation of NF-κB was strongly inhibited in the liver of LPS + FA-treated mice. FA alone did not exert any effect on the activation of NF-κB in our experimental model.

Ferulaldehyde diminished LPS-induced phosphorylation of MAPKs in RAW 264.7 macrophages

To study whether MAPKs were involved in the protective effect of FA in RAW macrophages, we measured phosphorylation of p38, ERK1/2 and JNK from control, LPS-, LPS + FA- and FA-treated cells 10 and 30 min after 100 ng/ml LPS challenge by Western-blotting utilizing phosphorylation-specific primary antibodies. Phosphorylation and thereby activation of all the kinases were increased following LPS stimulation at both time points (except for JNK at 10 min), that was attenuated by FA. FA alone did not exert any effect on the phosphorylation of the MAPKs.

Ferulaldehyde regulated LPS-induced MAPK activation via induction of MKP-1 expression

MKP-1 was reported to dephosphorylate all three MAP kinases. Furthermore, it was found to be a critical negative regulator in the innate immune response to LPS. In peritoneal macrophages induction of MKP-1 60 min after LPS treatment was reported, which was accompanied by attenuation of MAPK phosphorylation. However, MKP-1 was reported to have a fast turnover rate, therefore, we were interested how LPS and FA affected MKP-1 expression as early as 10 and 30 min after LPS treatment. In contrast to the previous notion that LPS monotonously induces MKP-1 expression, we found by Q-RT-PCR that MKP-1

mRNA level dropped to about half of the control value within 10 min after 100 ng/ml LPS application before increasing and reaching control level in 30 min. LPS-induced decrease of MKP-1 mRNA level was significantly diminished by FA 10 min after LPS treatment, and we found dramatically elevated MKP-1 mRNA expression levels in cells treated with FA alone or in combination with LPS at 30 min. MKP-1 protein concentrations reflected changes of its mRNA levels in a delayed fashion. Namely, we found markedly decreased MKP-1 protein concentration 30 min after 100 ng/ml LPS treatment that was prevented by FA, and elevated MKP-1 expression 60 min after LPS challenge. To summary, MAPK activation is followed by increased MKP-1 expression probably as a compensatory regulatory mechanism. In our *in vitro* experimental model, we found that FA shifted increased expression of MKP-1 forward in time which in turn attenuated activation of MAPKs.

Ferulaldehyde inhibited LPS-induced NF-κB activation in RAW 264.7 macrophages

Elevated ROS production and activation of MAP kinases in turn lead to nuclear translocation of NF-κB. In our experiments we were interested whether modulation of MAPK pathways by LPS and FA was reflected in NF-κB activation in RAW 264.7 macrophages. We assessed NF-κB activation in the cells by determining phosphorylation of its p65 subunit by Western blotting 10 min after 100 ng/ml LPS treatment, and accumulation of NF-κB-dependent expression of luciferase from an NF-κB-luciferase reporter plasmid for 24 h in 5 or 50 ng/ml LPS-treated macrophages. We found that even 5 ng/ml LPS induced a strong activation of NF-κB that was strongly attenuated by 50 μM FA. FA alone did not exert any effect on the activation of NF-κB in our *in vitro* model.

Direct free-radical scavenging activity of ferulaldehyde

Since we found that FA inhibited NO_2^- and ROS production induced by LPS plus IFN-γ in primary hepatocytes similarly to RAW macrophages, we determined whether the antioxidant property of FA was due to its free-radical scavenging activity. We tested direct free-radical scavenging activity of FA by measuring H_2O_2-induced oxidation of fluorescent redox dye dihydrorhodamine 123 in a cell-free *in vitro* system. FA at the concentration range of 5 to 100 μmol/L attenuated oxidation of the dye in a concentration dependent manner and it demonstrated a free-radical scavenging activity that was about the same as that of a known antioxidant, resveratrol. Published effects of resveratrol on cytokine profile, NF-κB translocation and kinase signaling were very similar to the effects of FA, furthermore, we

found that FA is present in red wine as a natural component or degradation product at a concentration comparable to resveratrol.

Conclusion

1. In our *in vivo* experiments we found that ferulaldehyde, a microbial end-product of several polyphenols has anti-inflammatory effect via decreasing early pro-inflammatory cytokines such as TNF-α, IL-1β and increasing the anti-inflammatory IL-10 in the sera of the LPS-treated mice. Additionally, FA inhibited LPS-induced activation of NF-κB transcription factor in the liver of mice. According to our data, these effects were probably due to attenuating LPS-induced activation of JNK and Akt. Furthermore, FA decreased ROS and RNS production in LPS plus IFN-γ-treated primary mouse hepatocytes, whose effects are expected to contribute to its anti-inflammatory property. These data provide the first direct *in vivo* evidence that a water soluble degradation product of polyphenols could be responsible for, or at least could significantly contribute to, the beneficial anti-inflammatory effects of polyphenol containing healthy foods, natural products and traditional medicines.

2. In our macrophage model we found that ferulaldehyde reduced ROS and RNS formations and protected mitochondria against LPS-induced rapid and massive membrane depolarization, which indicate the importance of mitochondrial integrity in early inflammatory response. Additionally, FA suppressed the activation of JNK, ERK and p38 MAPKs, thereby inhibited NF-κB activation in LPS-treated RAW 264.7 cells.

3. In our *in vitro* model, in contrast to the previous notion that LPS monotonously induces MKP-1 expression, we found that LPS induced early decrease of MKP-1 that was accompanied by activation of MAPKs. Additionally, we found that FA shifted elevated MKP-1 mRNA expression and protein levels forward in time which in turn attenuated activation of MAPKs in macrophage cells. All these data indicate importance of regulating MKP-1 expression and suggest it as a potential therapeutic target in early inflammatory processes.

4. Signaling mechanisms in the liver of mice and in macrophages showed a modified kinase activation pattern induced by the same type of LPS molecule. We found that FA inhibited LPS-induced JNK activation, but did not affect ERK1/2 and p38 MAPK pathways in liver of mice. However, unlike our *in vivo* model, FA attenuated LPS-induced activation of all three MAPKs except of ERK1/2 at 10 min suggesting a uniform regulation of MAPK activation in LPS-stimulated macrophages. These results may arise from the differences of the inflammatory models used, and cell- and tissue-specificity of the LPS-induced processes. Macrophages represent the first defense line against pathogens and are intensely exposed to the inflammation caused by infectious agents in this way they are involved in the induction of a stronger protective response to eliminate causative agents. However, liver which was the objective of our *in vivo* experiments, is a target organ for bacterial infection and as such it shows another type of protective response for pathogens.

Acknowledgement

This work was supported by Zoltan Magyary Research Fund and funds by Marie Curie European Re-Integration Grant (MERG-CT-2006-041964); OTKA F049515; OTKA K73738; GVOP-3.2.1-2004-04-0175/3.0; Hungarian Science Research Fund K-73738, F-67996; Innovation Research Team (TÁMOP-4.2.2-08/1/2008-0011); ETT 281/2009 and AOKKA 34039-1/2009.

I would like to thank prof. Dr. Balazs Sümegi and prof. Dr. Ferenc Gallyas Jr. the support, and guidance I received.

I would like to thank my colleagues dr. Balazs Radnai and dr. Balazs Veres for their cooperation, assistance and advices.

I would like to thank the technical help I recieved fom Helena Halász, Istvanne Pasztor, László Girán and Bertalan Horváth.

I would also like thank Prof. Dr. Kalman Hideg for synthesizing and conveying ferulaldehyde.

Publications

- Publications in the topic / A témában közölt publikációk -

Articles / Cikkek

Tucsek Z, Radnai B, Racz B, Debreceni B, Priber K J, Dolowschiak T, Palkovics T, Gallyas F Jr, Sumegi B, Veres B.: Suppressing LPS-induced early signal transduction in macrophages by a polyphenol degradation product: a critical role of MKP-1. J Leukoc Biol. 2010 Sep 30. [Epub ahead of print]
Impact factor: **4.403** (2009)

Citation: **0**

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.
Impact factor: **4.091**

Citation: **1**

Oral presentations /Előadások

Radnai Balázs, **Tucsek Zsuzsanna**, Bognár Zita, Antus Csenge, Márk László, Berente Zoltán, ifj. Gallyas Ferenc, Sümegi Balázs, Veres Balázs: Ferulaldehid, egy polifenol degradációs termék, gátolja az LPS-indukálta gyulladásos folyamatokat in vivo és in vitro. 40. Membrán-Transzport Konferencia, Sümeg, 2010. május 18-21.

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- Further publications / További publikációk -

Articles / Cikkek

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Impact factor: **4.254**

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