

[PC1-34] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Antioxidant actions of polyphenol QGR and its building blocks on unopsonized zymosan-induced oxidative stress in murine macrophages Raw264.7

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Polyphenol QGR (quercetin 3-O-beta-(2"-galloyl)-rhamnopyranoside) was isolated from *Persicaria lapathifolia* (Polygonaceae). In this study, we investigated inhibitory effects of QGR and its building blocks (quercitrin, quercetin and gallic acid) on superoxide anion and NO productions in unopsonized zymosan-stimulated murine macrophages Raw264.7. QGR and quercetin showed potent inhibitory effects on unopsonized zymosan-induced superoxide anion production with IC50 values of 3.2 μ M and 4.8 μ M, respectively. Quercitrin and gallic acid also inhibited the superoxide anion production with IC50 values of 30.9 μ M and 21.7 μ M, respectively. QGR showed scavenging effect on superoxide anion with an IC50 value of 56.1 μ M but quercetin did not exhibit. All of the polyphenols except quercetin did not show inhibitory effects on unopsonized zymosan-induced NO production. Furthermore, quercetin suppressed the expression of iNOS transcript in unopsonized zymosan-stimulated murine macrophages Raw264.7 but QGR and the other building blocks did not inhibit. Quercetin inhibited the NF- κ B transcriptional activity in a dose-dependent manner with 77.3% inhibition at 100 μ M, 52.6% at 39 μ M and 29.3% at 10 μ M.

[PC1-35] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Inhibitory Effect of the Extract from the Three Crude Drugs, *Kalopanax pictus*, *Pueraria thunbergiana* and *Rhus verniciflua* on NO and TNF- α production in LPS-induced Macrophage 264.7 Cells

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We previously reported the antimutagenicity, anti-lipid peroxidation and antiinflammatory properties of the three Chinese herb medicine, *Kalopanax pictus*, *Pueraria thunbergiana* and *Rhus verniciflua*, which have been used as therapeutics for diabetes mellitus and inflammation in Korea. Since these drugs have been used for the prescriptions consisted of several crude drugs, we investigated whether the extract of the three drugs could increase the individual drugs or not. The three extracts were independently prepared from *K. pictus*, *P. thunbergiana*, and *R. verniciflua*, which extracts were named the K-1, P-1 and R-1 in this order and the extract (KPR-1) was also prepared from the combined plant materials with each same weight of the three drugs. The three extracts, K-1, P-1 and R-1 were fractionated into the EtOAc extracts named K-2, P-2 and R-2 in this order. The extract of KPR-2 was also obtained from KPR-1 by the same way of fractionation. These eight samples were subjected to the nitrite assays in LPS-induced macrophage 264.7 cells and MTT assays. KPR-2 exhibited the most pronounced effect on the inhibition of NO production (IC₅₀: 5.1 mg/ml) but showed the weakest cytotoxicity with the value of IC₅₀ 434.5 mg/ml on the macrophage 264.7 cells among all the EtOAc extracts. It was evaluated that KPR-2 is a promising inhibitor of NO production, and it also significantly decreased PGE₂, and TNF- α release. Consistent with these observations, the expression NO synthase (iNOS) and cyclooxygenase-2 (COX-2) enzymes was significantly inhibited by KPR-2 in a concentration-dependent manner. These results suggested that the mixture extract and successive fractionation could lead to the better use of anti-inflammatory medicinal crude drugs.

[PC1-36] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Effects of Wogonin, a Plant Flavone from *Scutellaria Radix*, on Skin Inflammation: In Vivo Regulation of Inflammation-associated Gene Expression

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Flavonoids from plant origin show anti-inflammatory activity in vitro and in vivo. In addition to inhibition of inflammation-associated enzymes such as cyclooxygenases and lipoxygenases, they have been found to regulate the expression of inflammation-associated proteins from in vitro experiments. In order to prove in vivo behavior and the potential for beneficial use against inflammatory skin disorders, the effect of wogonin (5,7-dihydroxy-8-methoxyflavone) on in vivo expression of several inflammation-associated genes was examined in the intact as well as in the inflamed mouse skin by reverse transcriptase-polymerase chain reaction analysis. When applied topically on the intact skin, only a high dose treatment of wogonin (1000 mg/ear/3 days) slightly increased cyclooxygenase-1 and fibronectin m-RNA. On the other hand, wogonin at the doses of 250 – 1000 mg/ear/3 days potently lowered m-RNA levels of cyclooxygenase-2 and tumor necrosis factor- α with less effect on intercellular adhesion molecule-1 and interleukin-1 β in a sub-chronic skin inflammation model of tetradecanoylphorbol 13-acetate-induced ear edema (multiple treatment). The decrease of prostaglandin E₂ concentration (27.3 – 34.3%) was concomitantly observed in the wogonin-treated groups. A similar effect was also observed in an acute inflammation model of arachidonic acid-induced ear edema. From the present study, wogonin was proved to differentially regulate the expression of inflammation-associated genes in vivo and to become a useful therapeutic agent for skin inflammatory diseases mainly due to its modulation of the expression of proinflammatory molecules.

[PC1-37] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

The roles of ceramide on the cellular signal transduction in RAW 264.7 murine macrophages activated with lipopolysaccharide and interferon-gamma.

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Ceramide acts as a lipid second messenger in the cellular signal transduction and is involved in mediating a variety of cell functions such as proliferation, differentiation, growth arrest, and apoptosis. In the present study, we have investigated the effect of ceramide on cellular cytotoxicity and reactive oxygen species (ROS) to understand the relationship between them. Ceramide treatment significantly increased cell death in RAW 264.7 murine macrophages activated with lipopolysaccharide (LPS) and interferon-g (IFN-g). Interestingly, cotreatment with C₆-ceramide and LPS/IFN-g highly enhanced the production of ROS in cells. It is also shown that the production of NO and iNOS are inhibited by ceramide treatment in activated RAW cell, but the level of COX-2 was unaffected. The elevated level of ROS production was reduced by the presence of an NO donor S-nitroso-N-acetylpenicillamine (SNAP). These findings illustrate that the enhanced ROS generation may modulate the ceramide-mediated apoptosis and the cross-talk between NO and ROS-dependent transduction pathway will be possible. Understanding of the detailed mechanism will elucidate the pathway of ceramide-mediated cell death in murine macrophages.

[PC1-38] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Inhibition of nitric oxide and TNF- α production by propenone compound through blockaded of NF- κ B activation in cultured murine macrophages

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Lipopolysaccharide (LPS)-stimulated macrophages produced a large amounts of nitric oxide (NO) by inducible nitric oxide synthase (iNOS). This is an important mechanism in macrophages-induced septic shock and inflammation. In the present study, we tested a synthetic propenone compound, 1-furan-2-yl-3-pyridin-2-yl-propenone (FPP-3) for its ability to inhibit the production of tumor necrosis factor- α (TNF- α) and an inducible enzyme, iNOS, in the LPS-stimulated murine macrophage-like cell line, Raw264.7. FPP-3 consistently inhibited nitric oxide (NO) and TNF- α production in a dose dependent manner, with IC₅₀ values of 10.0 and 13.1 μ M, respectively. Western Blotting probed with specific anti-iNOS antibodies showed that the decrease in the quantity of the NO product was accompanied by a decrease in the iNOS