Three mistletoe lectins (ML-I, ML-IIU, ML-IIL) have been identified in Europe based on sugar specificities for galactose(Gal) and N-acetyl galactosamine(GalNAc). Korean mistletoe lectins have been known as mainly ML-II type. In previous results, we suggested that there are two lectins, 64 KDa and 60 KDa, in Korean mistletoe lectin (KML-C).
This paper describes a purification of two isolectins (referred to as KML-IIU, KML-IIL) from Korean mistletoe using immuno-affinity column generated from the KML-IIU-specific monoclonal antibody, biochemical and biological characterization of these proteins. Both lectins have two heterogeneous subunits and have carbohydrate-binding site that is specific for Gal/GalNAc but different in glycosylation, molecular weight and biological properties.
We found that the two lectins have similar amino acid compositions and similar level of affinity for galactose and N-acetylgalactosamine. However these lectins show different cytotoxic effects on various cells and different TNF-alpha inducing effects from macrophage.

[PC1-12] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Inhibition of lipopolysaccharide-induced inflammatory mediators NO, PGs, TNF-α expression by MeOH extract of Kochia scoparia in RAW264.7 cells.

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MeOH extract obtained from the Kochia scoparia (KS) was observed to inhibit tumor necrosis factor-alpha (TNF-alpha), prostaglandins (PGs) and nitric oxide(NO) production in a lipopolysaccharide (LPS)-stimulated murine macrophage cell line, RAW 264.7. These effects of MeOH-KS were based on modulation of iNOS and COX-2 level. Western blot analysis showed that MeOH-KS reduced the iNOS and COX-2 level in LPS activated macrophages, in a dose dependent manner without cNOS and COX-1 protein level. We also investigated RT-PCR to confirm the trancriptional regulation of iNOS and COX-2 mRNA by MeHO-KS.

[PC1-13] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Inhibitory effect of quercetin 3-O-β-(2"-galloyl)-rhamnopyranoside and its building moiety on the production of oxygen radicals in activated murine macrophages Raw264.7

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Reactive oxygen species play an important role in aging, carcinogenesis, and certain neurological disorders of human beings in addition to the host-defensive mechanism of inflammatory response. Murine macrophages Raw264.7 released superoxide anions via NADPH oxidase complex and nitric oxide (NO) via iNOS synthase when the cells were stimulated with unopsonized zymosan binding to complement receptor. Quercetin 3-O-β-(2"-galloyl)-rhamnopyranoside (QGR) showed dose-dependent inhibitory effects of 87% inhibition at 10 uM, 49% at 3 uM and 7% inhibition at 1 uM, and exhibited an IC50 value of 3 uM on the production of superoxide anions. Building moieties of QGR also showed inhibitory effects with IC50 values of 31 uM by quercitrin, 5 uM by quercetin and 22 uM by gallic acid on the unopsonized zymosan-induced production of superoxide anions. Murine macrophages Raw264.7 also released superoxide anions via NADPH oxidase complex when the cells were stimulated with phorbol myristate acetate (PMA) known as an activator of protein kinase C. QGR showed dose-dependent inhibitory effects of 86% inhibition at 30 uM, 67% at 10 uM, 45% at 3 uM and 23% at

1 uM, and exhibited an IC50 value of 5 uM on the PMA-induced production of superoxide anions. Building moieties of QGR also showed inhibitory effects with IC50 values of 3 uM by quercetin and 82-89 uM by quercitrin and gallic acid on the production of superoxide anions in PMA-stimualted murine macropahges Raw264.7. Quercetin has been reported to show inhibitory effects on several proinflammatory mediators, and its glycosides reduced the anti-inflammatory potency. Quercetin showed potent inhibitory effect on the production of superoxide anions. Similarly, inhibitory potency on the production of superoxide anions was reduced by quercitrin, but retained by QGR, a quercitrin gallate.

[PC1-14] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Acteoside induce antiproliferation and differentiation on HL-60, Human leukemia cell line, by cell cycle arrest.

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We investigated the in vitro effect of Acteoside , phenylpropanoid glycosides, is a natural product isolated from ···, on proliferation, differentiation and cell cycle regulation in human promyelocytic HL-60 leukemia cells. Acteoside significantly inhibited the proliferation of HL-60 cells, with IC50 of about 30ug/ml. It was also found to be a potent inducer of differentiation in human leukemia derived HL-60 cells through the examination of differentiation markers, as assessed by the reduction of nitroblue tetrazolium, the increase in esterase activities and phagocytic activity, and the expression of CD14 and CD66b surface antigens. Because a hallmark of terminal differentiation is the result of irreversible arrest in the G0/G1 or G2/M phase of the cell cycle, we investigated the effect of acteoside on cell cycle progression. To address the mechanism of the antiproliferative effect of acteoside, we investigated the effect of acteoside on cell cycle-related proteins in HL-60 cells. Acteoside did not change the steady-state levels of CDK4 and cyclinD3, but decreased the level of CDK2, CDK6 and cyclin D1, cyclin D2 , cyclin E . Hypophosphorylation of Rb protein was increased. The protein level of p21,p27 and p16, CDK inhibitor, were markedly increased and the mRNA level of p21 was also increased. In addition, acteoside markedly enhanced the binding of p21 with CDK6 compared with untreated control cells. In conclusion, the onset of acteoside-induced differentiation of HL-60 is linked to a sharp up-regulation of p21 level and a decrease in CDK6 activities. This is the first report that acteoside potently inhibit the proliferation of human promyelocytic HL-60 cells via differentiation.

[PC1-15] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Antitumor activity of organic compounds isolated from Korean mistletoe

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Velutin and betulinic acid were isolated as a cytotoxic principle from the dichloromethane extract of Korean mistletoe (Viscum album var. coloratum) by repeated silicagel chromatography and recrystallization. In in vitro analysis of cytotoxic activity using NIH-3T3 cells, dichloromethane extract of Korean mistletoe was shown to be highly cytotoxic against tumor cells. And we