P6.01.15

Inhibition of matrix metalloproteinase-9 activity in a leukemic cell line by Mentha spicata extract in vitro

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Introduction: Angiogenesis has an important role in cancer growth and metastasis. Matrix metalloproteinases (MMPs) are a large family of proteases degredate the extracellular matrix and have a significant role in tumor angiogenesis. The essential role of angiogenesis in leukemic patients has been reported. Current therapeutic methods in leukemia have not been very successful. Medicinal plants are widely used in treatment of cancers. Mentha spicata is a herb with known anti-tumor activities. In the present study, the effect of aqueous extract of Mentha spicata on MMP-9 activity in leukemic U937 cells has been assessed in vitro.
Materials and methods: U937 cells were cultured in RPMI with 10% FBS. Then the cells at logarithmic growth phase were incubated with different concentrations of aqueous extract of Mentha spicata (0.1 - 10 mg/ml) in the presence or absence of phorbol 12-myristate 13-acetate (PMA) (25 ng/ml) for 24, 48 and 72 hours. The MMP-9 activity in conditioned medium was evaluated by gelatin zymography.
Results: Mentha spicata aqueous extract significantly decreased the PMA-induced MMP-9 activity in leukemic U937 cells dose and time dependently.
Conclusions: The aqueous extract of Mentha spicata showed inhibitory effect on MMP-9 activity in U937 leukemia cells. So the anti-tumor effects of Mentha spicata may be in part due to its inhibitory effects on MMP-9 activity and consequently angiogenesis. Therefore Mentha spicata might have potential implication in leukemia treatment. Identification the effective element (s) in the Mentha spicata extract with anti-MMP activity could be valuable in designing new natural MMP suppressors.

P6.01.16

Labdane-type diterpene, constituent from myoga which are traditional Japanese food inhibit enzyme activity and gene expression of indoleamine 2,3-dioxygenase 1

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The activity of indoleamine 2,3-dioxygenase 1 (IDO-1), that catalyzes the degradation of tryptophan (Trp) into kynurenine (Kyn) increases after various inflammatory diseases. IDO-1 has an important immunomodulatory function in certain immune-related diseases.
We have developed a cell-based assay to evaluate the suppressive effect of phytochemicals or plant extracts on IDO-1 expression. When stimulated by INF-γ and LPS, profound high expressions of IDO-1 mRNA were detected in human monocyte THP-1 cells and were verified by quantitative real-time PCR.
Twenty two kinds of plants extracts and eleven kinds of phytochemicals were examined by the cell-based assay. As a result, the methanol extracts of Myoga flower buds which are traditional Japanese foods, and labdane-type diterpene galanal derived from the Myoga flowers significantly suppressed IDO-1 expression. The experiments using IDO-1-transfected HEK293 cells clarified that galanal inhibits not only the gene expression but also the enzyme activity. Galanal inhibited IDO-1 enzyme activity in a concentration-dependent manner with an IC_{50} value of 7.7 μM. The Lineweaver-Burk plot analysis indicated competitive inhibition of recombinant IDO-1 by galanal. Galanal decreased the Kyn concentration and recovered the Trp concentration in THP-1 cells stimulated with IFN-γ and LPS. Galanal suppressed the expression levels of pro-inflammatory cytokines. Interestingly, galanal up-regulate IFN-β, IL-2 and IL-3 gene expression levels. Galanal induced apoptosis in T cells by reduction of the Bcl-2: Bax ratio. Because the inhibitory activity of galanal is stronger than 1-methyl Trp which is a Trp analog, galanal may have a great potential as the novel drug for various immune-related diseases.

P6.01.17

HemoHIM, a novel composition of medicinal herbs, restores the lasting imbalance of Th1- and Th2-related immune response in *gamma*-irradiated mice and in aged mice

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Whole body irradiation brings down-regulation of the Th1-like immune response, resulting in an immunological imbalance for the long term. In ageing, one of serious problems is a shift towards a dominance of Th2 related response. HemoHIM, a mixture of 3 edible herbs, had been previously developed to protect the self-renewal tissues and promote the recovery of immune system against acute radiation syndrome. The current study was to evaluate the possibility of HemoHIM to restore the lasting immunological imbalance in γ-irradiated mice and in aged mice. The mice were exposed to γ-rays twice a week for a total dose of 5Gy. 12-month-old mice were used as aged mice. The irradiated mice were administrated orally with HemoHIM (100 mg/kg BW) from 1 week before the first irradiation for 4 or 6 months. The aged mice were intubated with HemoHIM for 3 weeks. HemoHIM ameliorated the lasting imbalance between Th1- and Th2-related immune responses that showed up in irradiated mice and in aged mice. In irradiated mice and in aged mice, HemoHIM restored the NK cell activities despite not changing the NK cell percentages. Moreover, the lasting low levels of IL-12p70 in irradiated mice and in aged mice were ameliorated by administrating HemoHIM. HemoHIM enhanced the phosphorylation of STAT4 that was decreased in the irradiated mice. Our findings showed that HemoHIM can ameliorate the lasting down-regulation of Th1-like immune responses by modulating IL-12p70/pSTAT4 signaling pathway in irradiated mice and in aged mice.

P6.01.18

Implications of the O-GlcNAc modification in the regulation of nuclear apoptosis in T cells

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O-linked β-N-acetylglucosamine (O-GlcNAc) is a nutrient-/stress-sensitive post-translational modification that affects nucleocytoplasmic proteins. Glucose, glucosamine and glutamine are the three major carbon and nitrogen donors for UDP-GlcNAc production. O-GlcNAcylation plays a role in fundamental regulatory mechanisms through the modification of proteins involved in cell division, metabolism, transcription, cell signaling and apoptosis. The aim of our research is to determine the implications of O-GlcNAc on T cell apoptosis. Human T lymphoblastic HPB-ALL cells were treated with an O-N-acetylglucosaminidase inhibitor (PUGNAc) or with glucosamine (GlcN) to increase O-GlcNAcylation. Apoptosis was induced in the presence of tributyltin (TBT) and DNA fragmentation was observed by cell cycle analysis. O-GlcNAcylated proteins were precipitated using succinylated wheat germ agglutinin (sWGA) for further western-blot identification. Our results showed that HPB-ALL cells treated with PUGNAc displayed a significant reduction in DNA fragmentation after TBT-induced apoptosis. DFF45, the protein that inhibits the endonuclease DFF40 in the heteromeric DNA fragmentation factor (DFF) complex, was identified as an O-GlcNAcylated protein. This modification appears to give DFF45 a stronger resistance to caspase cleavage during apoptosis. O-GlcNAcylation of DFF45 may represent a mechanism to control the accidental activation of DFF. Several publications in the last decade have shown a similar protective role of O-GlcNAc against different kinds of stress. Previous work from our laboratory showed that an enteral supplementation of glutamine helped reduce the morbidity and mortality of burn patients. The next step in our research will be to investigate the role of O-GlcNAc modifications in this protection.