

and three genes from *Enterococcus faecalis* V583 were predicted to encode homologs of the β -ketoacyl-acyl carrier protein synthases I or II or III of *Escherichia coli* (FabB or FabF, or FabH) were identified in the genomic database. The protein products were expressed, purified, and biochemically characterized. eFabH and hFabH carried out the initial condensation reaction of fatty acid biosynthesis with acetyl-Coenzyme A as a primer, and hFabB and eFabF1 carried out the elongation condensation reaction of fatty acid biosynthesis with myristoyl-ACP.

[PC1-12] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Purification and Characterization of Dermatan Sulfate from Eel Skin, *Anguilla japonica*

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Dermatan sulfate (DS) was isolated from eel skin (*Anguilla japonica*) by actinase and endonuclease digestions followed by β -elimination reaction and DEAE-Sephacel chromatography. DS was a major glycosaminoglycan in eel skin with 88% of the total uronic acid. The content of IdoA2S α 1 \rightarrow 4GalNAc4S sequence in eel skin, which is known to be a binding site to heparin cofactor II, was two times higher than that of dermatan sulfate from porcine skin. The anti-IIIa activity of eel skin dermatan sulfate mediated through heparin cofactor II (HCII) was 25 units/mg, whereas DS from porcine skin shows 23.2 units/mg. The average molecular weight was determined as 14 kDa by gel chromatography on a TSKgel G3000SWXL column. Based on H1 NMR spectroscopy, we suggest that 3-sulfated and/or 2,3-sulfated IdoA residues are present in the chain.

[PC1-13] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Induction of apoptosis in human promyelocytic leukaemia HL-60 cells by manassatin B involves release of cytochrome c and activation of caspases

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Manassantin B classified into dineolignans have been isolated from *Saururus chinensis* Manassantin B was found to induce apoptosis in human promyelocytic leukaemia HL-60 cells with characteristic apoptotic features like increase of nucleosomal ladder, apoptotic body formation, flipping of membrane phosphatidylserine. Manassantin B induced FAS and FAS ligand expression, and activated caspase 8 which cleaved bid to tbid in cytosol. The release of cytochrome c to cytosol was accompanied with decrease of bcl-2 protein and increase of tbid and bax protein in mitochondria. Released cytochrome c activated caspase 9 and -3, but these effects were completely attenuated by the treatment of broad caspase inhibitor, Z-VAD fmk. These results indicate that manassatin B induce apoptosis through upregulation of FAS, caspase family and mitochondria -related proteins.

[PC1-14] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Induction of Differentiation in HL-60 Human leukemia cells by Acteoside.

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In previous reports, we exhibited that acteoside showed significant cytotoxicity against various cancer cells. In this study we investigated that acteoside is capable of inducing differentiation in HL-60 human leukemia cell line. After being treated with acteoside, the growth curve was decreased remarkably in a dose- and time-dependent manner, and cell doubling time was delayed. Exposure of cells to 20 μ g/ml acteoside induced differentiation of HL-60 cells to monocyte/macrophage-like cells by cell surface antigen expression. The percentage of NBT reducing activity was increased in a time-dependent manner. In addition, the protein level of p21 and p16 increased and ppRb decreased in western blot analysis. These results suggest that acteoside possess the activity of inducing differentiation in HL-60 cells.

[PC1-15] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

An investigation of the effect of epigallocatechin-3-gallate on the renal dipeptidase release

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The action of epigallocatechin-3-gallate (EGCG), polyphenol compound from green tea, on the release pattern of glycosylphosphatidylinositol (GPI)-anchored renal dipeptidase (RDPase) from renal proximal tubules (PTs) was examined. EGCG had a stronger inhibitory effect on the release of RDPase than alkaline phosphatase (APase), another GPI-anchored ectoenzyme used as a reference protein. The effect of EGCG on cell viability as assessed by MTT test was found to be intact, and moreover, was indicative of potent cell activation or proliferation. Inhibition of RDPase release by EGCG was also confirmed by time-dependent manner. The concentration of nitric oxide (NO), measured by nitrite, in the incubation supernatant increased up to 6-fold at 100mM EGCG, whereas the release of RDPase decreased in inverse proportion less than 10% of the control, thus demonstrating the inhibitory effect of EGCG on the release of RDPase via NO production. We investigated whether the inhibitory effect of EGCG by NO production was affected by NO-dependent downstream cGMP signaling with the specific inhibitors of the NO-soluble guanylate cyclase pathway, [1H-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one] (ODQ) and the inhibitor of cGMP-selective phosphodiesterase, Zaprinast. However, there was no evidence of cGMP involvement and also of intracellular Ca²⁺ concentration in inhibition of RDPase release by EGCG. When the supernatant PLC by incubation PTs was treated to PTs with EGCG for 15min, the marked increase of RDPase release was strongly blocked. These data support that the inhibitory action of EGCG on the RDPase release was mainly determined by an interference of external PI-PLC reaction rather than cellular signaling stimulated by NO.

[PC1-16] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Peroxynitrite Scavenging Activity of Active Constituents from *Scutellaria baicalensis*

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Peroxynitrite, formed from the reaction of .O₂- and .NO, is a cytotoxic species that can oxidize several cellular components such as proteins, lipids and DNA. Oxidative stress is considered to be the major cause of aging and many age-related diseases including Alzheimer's disease, rheumatoid arthritis, cancer, and atherosclerosis. ONOO-, a powerful oxidant, can cause damage of proteins, lipid and DNA through nitration and oxidation. The aim of this study was to evaluate of the ability of methanolic extract and fractions from *Scutellaria baicalensis* and to screen the active components. Methanolic extract showed strong ONOO- scavenging activity. Among fractions, ethylacetate fraction had the potent scavenging activity. In further analysis, baicalin, 5,7,2',5'-tetrahydroxy-8,6'- dimethoxyflavone(TDF), wogon, baicalein, rhapontigenin and rhaponticin were identified from fractionated extract. Results showed that the most effective compounds were baicalin and TDF which led to decreased ONOO--mediated nitration of tyrosine through electron nitration and showed significant inhibition on nitration of albumin and GSH reductase by ONOO- in a dose-dependent manner. TDF and baicalin can be developed as effective ONOO- scavengers for the prevention of ONOO--involved diseases.