Cytotoxicity and apoptosis inducing effects of phenylpropanoids.

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This study was carried out to determine cytotoxicity and apoptosis inducing effects of the seven pheylpropanoids. We have determined cytotoxocity by MTT(3-(4,5-dimethyl-thiazol-2-yl)-2,5-dipheryl-2H-tetrazo-liumbromide)assay and investigated the extendency of apoptosis by DAPI assay. The IC50s (ug/ml) of each phenylpropanoids by MTT(HL-60 cell) are 10.3(KYS50046), 15.9(KYS50047), >100 (KYS50049), >100 (KYS50050), 50.9(KYS50051), 52.3(KYS50153) and 48.36(KYS50154). Based on this result KYS50046 and KYS50047 showed significant cytotoxicity. When we observe Structure-activity relationship(SAR) of these compounds, KYS50049-51 which are unefficient in the cytotoxicity have methoxy group instead of hydroxy group on the phenol ring (ortho-form) compared to KYS50046-7. There is no sugar ring in KYS50153-4 which are also unefficient in cytotoxicity, whereas KYS50046-7 have sugar ring on the ring. So we suppose that the existences of hydroxy group on the phenol ring and sugar ring are *essential moiety* for cytotoxicity and apoptosis inducing effects.

[PC1-22] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Hydroquinone from Coffee Modulates Reactivity of Peroxynitrite and Nitric Oxide Production

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Peroxynitrite (ONOO-), a potent cytotoxic oxidant formed by the reaction of nitric oxide (.NO) and superoxide radical (.O2-), may be rapidly lethal in a cellular mileu if left unchecked due to oxidization and nitration process. In the present study, we investigated ONOO- scavenging effect of hydroquinone from coffee, consisting of both beneficial and hazardous biochemical substances, and its biological effect on NO metabolism in the murine macrophage RAW 264.7.
Hydroquinone strongly scavenged ONOO--induced dihydrorhodamine 123 (DHR123) oxidation not by electron donation but by nitration of the compound itself in a dose-dependent manner. The compound also decreased ONOO--induced nitrotyrosine of GSH reductase, suggesting that hydroquinone directly neutralizes ONOO- and may prevent ONOO--induced damage of GSH reductase. Furthermore, hydroquinone also suppressed NO production, which is one of upstream sources via inhibition of inducible NO synthase (iNOS) expression in lipopolysaccharide (LPS)-activated RAW 264.7 cells. The inhibitory effect of hydroquinone was mediated through blocking LPS-induced signaling pathway, since hydroquinone potently inhibited nuclear factor-kB (NF-kB), and phosphorylation of extracellular signal related kinases 1 and 2 (ERK 1/2), a member of the MAPK family. Our results suggest that hydroquinone may be regarded as a potent regulator of ONOO--mediated diseases via both directly scavenging and indirectly blocking ONOO- production pathways, such as NO synthesis.

[PC1-23] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Long-term Evaluation of the Mouse Model for Validity of Studying the Effects of Helicobacter pylori Infection on Gastric Carcinogenesis

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This study was aimed at evaluating the effect of *H. pylori* infection on gastric carcinogenesis. Four-week-old pathogen free C57BL/6 mice (n=115) were infected with SS1, the mouse-adapted *H. pylori* strain. *H.*

pylori-infected mice were sacrificed 4, 8, 16, 24, 36, 50 and 80 weeks after the bacterial infection. After 80 weeks of infection, almost all *H. pylori*-infected mice developed hyperplastic or atrophic gastritis, but did not show any evidence of adenoma, dysplasia or carcinoma. PCNA positive cells were the most abundant 50 weeks after the *H. pylori* infection, but their number decreased thereafter up to 80 weeks. Apoptotic cell death became evident 8 weeks after *H. pylori* infection, with 7-8 apoptotic cells/high power field, and increased thereafter. Production of normally observed neutral muscin was decreased gradually, with maximal reduction observed 50 weeks after *H. pylori* infection, which was accompanied by acid chracterization thereafter. The SS1 infected mouse seems to be a suitable animal model for *H. pylori*-related research, although *H. pylori* infection itself does not induce gastric cancer in a long-term normal wild-type mouse model, which could be explained by the balance between cell proliferation and apoptosis.

[PC1-24] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Effects of chitosan on renal dipeptidase in vivo

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Urinary dipeptidase (Udpase) which was detected in urine of various animals including rat, rabbit, pig and human is a released form of renal dipeptidase (RDPase, EC 3.4.13.19), a glycosylphosphatidylinositol (GPI) linked ectoenzyme of renal proximal tubules. Udpase activity was decreased in urine samples of acute and chronic renal failure patients. This study was undertaken to examine the effect of chitosan, a deacetylated derivative of chitin, on RDPase from rat kidney in vivo. Rats were fed with chitosan (0.1% in distilled water) for 3, 8 and 15 months and the urine samples were collected followed by ammonium sulfate precipitation (50~75% saturation). The activity of Udpase was measured with the modified fluorometric method of Ito et al (1984) and the protein was related with band intensity of SDS-electrophoresis. Approximately 4-folds Increase of Udpase was observed in the chitosan fed rats for 3 months and such increase was less prominent in rats of 12 months or older. These results suggest that chitosan may elevate the renal function and speed up the recovery from renal failure, or even prevent the renal diseases.

[PC1-25] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Genetic structure responsible for catechol catabolism in P.cepacia G4

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The meta-cleavage pathway for catechol is one of the major routes for the microbial degradation of aromatic compounds. Pseudomonas cepacia G4 grows efficiently on toluene, cresol, or phenol via a plasmid-encoded catechol 2,3-dioxygenase and a subsequent meta-cleavage pathway. A recombinant plasmid pCNU 301 is a 23.0-kb BamHI restriction endonuclease-cleaved DNA fragment cloned from the chromosome of Pseudomonas cepacia G4. The pCNU 301 contains tomBCEGFD gene cluster which can encode six enzymes catabolizing catechol to acetyl CoA. In this study nucleotide sequences of tomBCEGFD gene encoding catechol-2,3-dioxygenase(C23O), 2-hydroxymuconic semialdehyde dehydrogenase (HMSD), 2-hydroxypenta 2,4-dienoate hydratase(HPDH), acetaldehyde dehydrogenase(ADH), 4-hydroxy-2-oxovalerate aldolase, and 4-oxalocrotonate decarboxylase were determined. The catechol 2,3-dioxygenase gene(tomB) was consisted of 945 bases. Amino acid sequence of the tomB gene product exhibited 82% identity with that of catechol 2,3-dioxygenase from P. putida UCC2. The 2-hydroxymuconic semialdehyde dehydrogenase gene(tomC) was consisted of 1458 bases. Amino acid sequence of the tomC gene product exhibited 78% identity with that of 2-hydroxymuconic semialdehyde dehydrogenase from P. putida CF600. The 2-hydroxypenta 2,4-dienoate hydratase gene(tomE) was consisted of 783 bases. Amino acid sequence of the tomE gene product exhibited 81% identity with those of 2-hydroxypenta 2,4-dienoate hydratase from Pseudomonas sp. LB400 and P. pseudoalcaligens KF707. The acetaldehyde dehydrogenase gene(tomG) was consisted of 912 bases. Amino acid sequence of the tomG gene product exhibited 88% identity with those of acetaldehyde dehydrogenase from Pseudomonas sp. LB400 and P. pseudoalcaligens KF707. The 4-hydroxy-2-oxovalerate aldolase gene(tomF) was consisted of 1047 bases. Amino acid sequence of the tomF gene product exhibited 87% identity with that of 4-hydroxy-2-oxovalerate aldolase from Comamonas testosteroni TA441. The 4-oxalocrotonate decarboxylase gene(tomD) was consisted of