Glutathione Reductase

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Peroxynitrite, a potent cytotoxic oxidant formed by the reaction of nitric oxide(NO) with the superoxide anion radical(O2.-), reacts rapidly to cause of highly toxic oxidizing and nitrating process. The importance of the regulation of ONOO- has been recently recognized because of lack of specific endogenous anti-oxidative defense enzyme against ONOO-. This lack of defense necessitates search for the exogenous source of effective scavengers against ONOO-. Coffee is a complex mixture containing a variety of compounds. The mutagenic effects of instant coffee have been reported and its constituents are known free radical generators, damaging DNA, lipid and protein. In the present study, we report on the anti-oxidative effect of coffee ingredients. Our major effort was on the scavenging effect on peroxynitrite. At present, there is no information available on peroxynitrite scavenging activity of coffee ingredients. We investigated the protective effect of coffee ingredients against peroxynitrite using GSH reductase whose activity is dependent on the integrity of tyrosine. The effectiveness of protection was monitored by the prevention of protein nitration by peroxynitrite. In the study, we focussed on two major coffee components: hydroquinone and 3-methyl-1, 2-cyclopentadione. The scavenging activity of two coffee ingredients was assessed by following three methods: 1) the quantitation of the oxidation of dihydrorhodamine 123 to rhodamine 123. 2) inactivation of GSH reductase activity and 3) tyrosine nitration of GSH reductase by Western blot analysis.
Results showed that hydroquinone and 3-methyl-1, 2-cyclopentadione effectively suppressed peroxynitrite-mediated tyrosine nitration of glutathione reductase in a dose-dependent manner. The extent of prevention of nitration was reflected on GSH reductase activity, indicating the protection of tyrosine moiety in the enzyme. Data are further confirmed by level of tyrosine nitration in GSH reductase analyzed by Western blot analysis.
To our knowledge, this is the first report on the peroxynitrite scavenging action by hydroquinone and 3-methyl-1, 2-cyclopentadione in coffee. Reaction mechanisms of these active ingredients against peroxynitrite need further elucidation.

[PA2-2] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Hamamelitannin as a peroxynitrite scavenger

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Peroxynitrite (ONOO-), formed in vivo from the reaction of superoxide ('O2-) with nitric oxide ('NO), is a cytotoxic species that can oxidize several cellular components such as proteins, lipids, and DNA and nitrate many amino acids, including tyrosine. The purpose of our present study was to investigate the protective effect of hamamelitannin, the major active component isolated from witch hazel (Hamamelis virginiana L.) bark, against damages induced by ONOO-. Hamamelitannin (IC50=1.05μM) exhibited very potent ONOO- scavenging activity measured by oxidation of dihydrorhodamine 123 with fluorescence method. Our data suggest that hamamelitannin led to decrease ONOO--mediated nitration of tyrosine by its electron donation in spectrophotometric analysis. Using immunoassay, hamamelitannin showed the significant inhibition on nitration of bovine serum albumin (BSA) and low-density lipoprotein (LDL) by ONOO- in a dose dependent manner and the relative inhibitory effect on oxidation of BSA and LDL by ONOO-. Hamamelitannin also provided protection against cell damage mediated by ONOO-. In conclusion, our results suggest that hamamelitannin can be developed as an effective supplementary antioxidant in peroxynitrite toxicity.

[PA3-1] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Doxylamine Concentration in Blood and Tissues of Rats after the Oral Administration

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As fatal cases by doxylamine overdose are increased, we tried to analyze the concentration of doxylamine in tissues and blood of rats. There is two kind of death cases which related to the doxylamine overdose. The one is pretreated with common dose of doxylamine and the other one is non-pretreated. So we also separated rats with two groups and compared with each other. The first group of rats pretreated with common dose(5mg/kg) for a week but the second group was not pretreated. At 8th day of experiment, we orally administered to rats with doxylamine overdose (750mg/kg) which value is more than LD50, 500mg/kg.
When the rats were alived, then they were sacrificed with ether. Isolated tissues were liver, lung, brain, kidney and spleen. Tissues and blood were compared between two group.
After all, doxylamine concentration in tissues and blood of first group was higher than those of second group. But tissue distribution of doxylamine concentration has difference among tissues. The order of doxylamine concentration was liver> kidney> spleen≥lung> brain in first group. On the contrary in second group the order was kidney> lung≥
spleen> liver> brain.

[PA3-2] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl,.Bldg 3]]

The inhibitory effects of hypoxic condition and nitric oxide on dioxin stimulated endogenous CYP1A1 activity in Hepa I cells

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In order to understand the effects of hypoxic agents and nitric oxide on endogenous CYP1A1 activity in Hepa I cells, the ethoxyresorufin-O-dealkylase (EROD) activity was determined. When 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) administered into Hepa I cells, EROD activity was induced in dose dependent manner. SNP (Sodium nitroprusside), Which donates nitric oxide, inhibited TCDD stimulated EROD activity. And LPS (lipopolysaccharide), which induces iNOS, also inhibited TCDD stimulated EROD activity. NG-nitro-l-arginine, the inhibitor of iNOS, recovered the TCDD stimulated induction suppressed by LPS, and this effect was abolished when the substrate of iNOS, l-Arginine was administered concomitantly. To mimic hypoxic condition, cobalt chloride, picolinic acid and desferrioxamine were administered into Hepa I cells. When the iron chelating agents, such as picolinic acid, desferrioxamine, were administered concomitantly with TCDD, TCDD stimulated EROD activity was reduced in dose dependent manner. Cobalt chloride known as hypoxia inducing agent also inhibited TCDD stimulated EROD activity. These data shows that hypoxic agents and nitric oxide inhibit TCDD stimulated endogenous CYP1A1 activity in Hepa I cells.

[PA3-3] [04/21/2000 (Fri) 10:30 - 11:30 / [1st Fl, Bldg 3]]

Anti-platelet activity by specific thrombin inhibitor, LB30057

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The effect of LB30057, a synthetic compound, on platelet activity and its mechanism of action was