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Title	Characterization of Liver Cytochrome P450 Subfamilies in Seals from the Coast of Hokkaido and Factors Responsible for Their Activities and Contents
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anticancer drug-induced apoptosis. In MOLT-4 cells exposed to 15Gy of X-ray, DNA fragments were detected by agarose gel electrophoresis 12 h after X irradiation. This DNA fragmentation was inhibited by not only a metabolic product of ceramide, sphingosine-1-phosphate, but also a specific inhibitor of caspase-3 (Ac-DEVD-CHO). Western blot analysis showed that the phosphorylated active form of SAPK/JNK appeared 1 h after X irradiation and its concentration was maintained at the constant level for at least 9 h. The active form of caspase-3 (p20) was detected 3 h after X irradiation and the subsequent cleavage of nuclear enzyme poly (ADP-ribose) polymerase by p20 were detected. Furthermore, a cell permeant $[Ca^{2+}]_i$ chelator, (acetyoxymethyl-) 1, 2-bis (*o*-aminophenoxy) ethane-*N*, *N*, *N'*, *N'*-tetraacetic acid, gave the lag in the onset of the activation of SAPK/JNK and caspase-3 as well as the DNA fragmentation, but this compound gave no effect on the X-ray-induced accumulation of aberrant p53.

These results obtained from p53-unfunctional MOLT-4 cells demonstrated that X irradiation induced the increase of $[Ca^{2+}]_i$ which became a trigger of the activation of SAPK/JNK and caspase-3 and produced the ladder-like DNA fragments. These data may provide useful information about the radiation treatment of p53-unfunctional tumors.

Characterization of Liver Cytochrome P450 Subfamilies in Seals from the Coast of Hokkaido and Factors Responsible for Their Activities and Contents

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In order to understand species-specific characterization of liver microsomal cytochrome P450 subfamilies in two species of seals, Phoca largha and Phoca fasciata, and to elucidate factors responsible for their enzymatic activities and contents, the P450 spectrum, P450-dependent enzyme activity and Western blotting analysis using fresh livers of seals were studied. The results and discussion in this study can be summarized as follows. (1) Dithionite difference spectra of CO-treated seal microsomes showed two peaks around 448nm and 420nm. Appearance of the peak at around 420nm indicates that sample degradation occurred during storage conditions prior to microsome preparation. (2) Alkoxyresorufin O-dealkylase (AROD) and testosterone hydroxylase (TH) activities were detected in both seal species although not in some samples. Seal microsomes could metabolize ethoxyresorufin at a higher rate than other alkoxyresorufins compared to the rat microsomes. In contrast to rat, seal microsomes could hydroxylate testosterone at 2β-, and 16 β-positions, although 2α, 7α, 16α-hydroxy testosterone and androstenedione were not produced. (3) Western blotting analysis showed the presence of Rat CYP1A1-, 1A2-, 2B (single band detected)-, and 3A (two bands detected)-like proteins in both seals. The CYP2B-like protein band in seal microsomes was apparently weaker than untreated rat CYP2B, suggesting that less CYP2B-like protein was produced or immunodetected by the rabbit anti-rat CYP2B1 antibody. (4) Correlations among AROD activi-

ties were significant. In addition, AROD and immunodetectable CYP1A-like protein were also significantly correlated. This indicates that the CYP1A-like protein is involved in the AROD activities. In P. largha, immunodetectable CYP3A-like protein was significantly correlated with AROD. This suggests that the CYP3A-like protein in this seal is also responsible for catalytic activities of AROD. Immunodetectable CYP2B-like protein was not correlated to any activities. The CYP2B-like protein may be unlikely to participate in the substrate metabolism. As immunodetectable CYP1A- and CYP3A-like proteins in P. largha were also correlated with TH activity, these CYP subfamilies may be associated with TH. (5) In P. largha, a growth-dependent increase of EROD activity was found in male seals. In contrast, female seals showed a decreasing pattern in EROD activity. Such specific patterns of EROD activity in male and female seals were found to be similar to those of organochlorine accumulation, implying a potential for induction of seal P450 by environmental pollutants.

Generation of Reactive Oxygen Species and Decrease of Liver Catalase in Long-Evans Cinnamon Rats Predisposed to Hepatitis and Hepatoma.

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The Long-Evans Cinnamon (LEC) rat, an inbred mutant rat derived from the Long-Evans strain, is characterized by spontaneous hepatitis due to gross accumulation of hepatic Cu. We have studied generation of reactive oxygen species, superoxide anions, hydrogen peroxide, and catalase, which could be a reduction enzyme of H2O2, in LEC and Wistar rat livers. Generation of superoxide anions was higher in the liver of female LEC rats than in that of Wistar rats at 14 wk (acute stage) of age. However, generation of superoxide anions was lower in the liver of male LEC rats than in that of Wistar rats at 16 (acute stage) and 24 wk (chronic hepatitis stage) of age. Generation of hydrogen peroxide was higher in the liver of LEC rats than in that of Wistar rats. Of the enzymes examined, hepatic catalase was markedly decreased in LEC rats. Western-blot analysis revealed that the content of catalase in the liver of LEC rats was 44%, 39%, 23%, and 26% of the control values at 9 (normal stage), 14 (acute hepatitis stage), 16 (acute hepatitis stage), 24 (chronic hepatitis stage) wk of age, respectively. These results suggest that cellular accumulation of ·OH from H2O2 in the presence of Cu, causes cellular damage leading to hepatitis and hepatoma.