P-33

Structural analysis of DNA adducts formed from *N*-nitrosotaurocholic acid (NO-TCA).

Y Totsuka[1], R Nishigaki[1], S Enomoto[1], T Takamura-Enya[1], K Masumura[2], T Nohmi[2], T Sugimura[1], and K Wakabayashi[1] (Natl Cancer Centr Res Inst[1], NIHS[2]) ytotsuka@gan2.res.ncc.go.jp

Keywords: *N*-nitrosotaurocholic acid; *N3*-ethanesulfonic acid -2'-deoxycitidine; N^4-cholyl-2'-deoxycitidine

N-Nitrosoglycocholic acid (NO-GCA) and *N*-nitrosotaurocholic acid (NO-TCA) have been reported to be mutagenic in *Salmonella* and carcinogenic in rats. It is also reported that DNA-alkylation, containing O^6-carboxymethyl-guanine, is observed *in vitro* when NO-GCA is incubated with calf thymus DNA. To investigate the formation of DNA adducts by NO-TCA, NO-TCA was incubated with calf thymus DNA, and adduct formation was analyzed by the ^{32}P-postlabeling method under nuclease P1 conditions. Three kinds of adducts were detected in calf thymus DNA treated with NO-TCA, and the major adduct was determined to be *N*3-ethanesulfonic acid -2'-deoxycytidine. In addition, two bulky adducts containing the bile acid moiety were also produced, and the structures were concluded to be N^4-cholyl-2'-deoxycytidine and N^6-cholyl-2'-deoxyadenosine, respectively. When NO-TCA was singly administered to male Wistar rats by gavage at a dose of 250 mg/kg, both ethanesulfonic acid-dC and N^4-cholyl-dC were detected in the glandular stomach and colon. The adduct levels of *N*3-ethanesulfonic acid-dC were 0.22 - 0.29 per 10^6 nucleotides, and values for N^4-cholyl-dC were about 500-fold lower. Since NO-TCA may be formed by nitrosation of taurocholic acid in the human body, the presence of *N*3-ethanesulfonic acid-dC and N^4-cholyl-dC are now being analyzed in human tissues.

N-ニトロソタウロコール酸より生成されるDNA付加体の構造解析
戸塚ゆ加里[1]、西垣玲奈[1]、榎本茂樹[1]、高村 (塩谷) 岳樹[1]、増村健一[2]、能美健彦[2]、杉村隆[1]、若林敬二[1] (国立がんセ・研・がん予防基礎[1]、国立医薬食衛生研[2])

P-34

Oxidative DNA damage induced by carcinogenic aromatic amines, *o*-anisidine and *o*-dianisidine

Y Hiraku, H Note and S Kawanishi (Mie University Graduate School of Medicine) y-hiraku@doc.medic.mie-u.ac.jp

Keywords: oxidative DNA damage; *o*-anisidine; *o*-dianisidine

o-Anisidine and *o*-dianisidine, which have been used as intermediates in the production of dyes, are urinary bladder carcinogens. We examined oxidative DNA damage induced by these aromatic amines in the presence of cytochrome P450 (CYP) using [^{32}P]-5'-end-labeled DNA fragments obtained from human cancer-relevant genes. *o*-Anisidine and *o*-dianisidine induced Cu(II)-mediated DNA damage in the presence of CYP1A2 and 2C9 to a greater extent than CYP 1A1, 2D6 and 2E1. *o*-Dianisidine induced DNA damage much more efficiently than *o*-anisidine in the presence of CYP1A2. CYP1A2-pretreated *o*-anisidine and *o*-dianisidine formed piperidine-labile and Fpg-sensitive lesions at cytosine and guanine residues of the 5'-ACG-3' sequence complementary to codon 273 (a mutational hotspot) of the *p53* tumor suppressor gene, respectively. DNA damage induced by these aromatic amines was inhibited by catalase and bathocuproine, suggesting that H_2O_2 and Cu(I) were involved. These results suggest that Cu(I)-hydroperoxo complex is the primary reactive species causing DNA damage. Formation of 8-oxo-7,8-dihydro 2'-deoxyguanosine, an indicator of oxidative DNA damage, was significantly increased by CYP1A2-treated *o*-anisidine and *o*-dianisidine in the presence of Cu(II). Therefore, it is concluded that *o*-anisidine and *o*-dianisidine may express the carcinogenicity through oxidative DNA damage induced by their metabolites.

発癌性芳香族アミン*o*-アニシジンおよび*o*-ジアニシジンによる酸化的DNA損傷
平工雄介、野手英明、川西正祐 (三重大学大学院医学系研究科)