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Metabolism and excretion of 1-hydroxymethylpyrene, the proximate metabolite of the carcinogen 1-methylpyrene, in rats

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ABSTRACT

1-Methylpyrene, an alkylated polycyclic aromatic hydrocarbon and environmental carcinogen, is activated by side-chain hydroxylation to 1-hydroxymethylpyrene (1-HMP) and subsequent sulfo conjugation to the DNA-reactive 1-sulfooxymethylpyrene. In addition to the bioactivation, processes of metabolic detoxification and transport greatly influence the genotoxicity of 1-methylpyrene. For a better understanding of 1-HMP detoxification in vivo we studied urinary and fecal metabolites in rats following intraperitoneal doses of 19.3 mg 1-HMP/kg body weight (5 rats) or the same dose containing 200 μCi [^{14}C]1-HMP/kg body weight (2 rats). After 48 h, 48.0% (rat 1) and 29.1% (rat 2) of the radioactivity was recovered as 1-HMP in the feces. Six major metabolites were observed by UV and on-line radioactivity detection in urine samples and feces after HPLC separation. The compounds were characterized by mass spectrometry, ^{1}H NMR and ^{1}H-^{1}H COSY NMR spectroscopy, which allowed assigning tentative molecular structures. Two prominent metabolites, 1-pyrene carboxylic acid (M-6) and the acyl glucuronide of 1-pyrene carboxylic acid (M-5) accounted for 17.7% (rat 1) and 25.2% (rat 2) of the overall radioactive dose. Further, we detected the acyl glucuronide of 6-hydroxy-1-pyrene carboxylic acid (M-1) and 8-sulfooxy-1-pyrene carboxylic acid (M-3) together with two regioisomers of M-3 (M-2 and M-4) differing in position of the sulfate group at the pyrene ring. In urine samples, the radioactivity of 1-pyrene carboxylic acid and its five derivatives amounted to 32.4% (rat 1) or 45.5% (rat 2) of the total [^{14}C]1-HMP dose.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are formed as a result of incomplete combustion of organic material and can be classified as purely aromatic and alkylated derivatives. 1-Methylpyrene (1-MP) is a prototype of an alkylated PAH. It is a common environmental carcinogen that has been found in cigarette and marijuana smoke (Bi et al., 2005; Husgafvel-Pursiainen et al., 1986; Lee et al., 1976; Severson et al., 1976), exhaust of diesel engines (Jensen and Hites, 1983). Levels of 1-MP at 4.1–36.2 μg/100 cigarettes were 3–10 times higher compared to those of benzo[*a*]pyrene in eight different brands of cigarettes (Grimmer 1979; Lee et al., 1976; Severson et al., 1976). 1-MP was also detected at concentrations similar to those of benzo[*a*]pyrene in restaurants and other locations with tobacco smoke exposure (1.3–8.0 ng/m^3) (Husgafvel-Pursiainen et al., 1986), in samples of smoked cheese (0.04–0.3 μg/kg) (Guillen and Sopelana, 2004), in olive oil (1.3–35 μg/l) (Guillen et al., 2004) and as bio-accumulated pollutant in marine tissues (Pancirov and Brown, 1977).

In contrast to pyrene, which is considered to be non-carcinogenic (IARC, 1983; Rice et al., 1988), 1-MP induced formation of hepatic tumors in newborn mice (Rice et al., 1987). This activity may originate from metabolic activation by benzylic hydroxylation and subsequent sulfo conjugation. The hydroxylation at the exocyclic carbon leads to formation of 1-hydroxymethylpyrene (1-HMP). It was observed in rat hepatic homogenates (Rice et al., 1988) and genetically engineered Chinese Hamster V79 cell lines expressing human (h) or rat (r) cytochromes P450 (CYP). From nine CYPs studied hCYP1A1, hCYP1B1 and

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rCYP1A1 contributed most to side-chain hydroxylation of 1-MP, whereas hCYP1A2, hCYP2A3, hCYP3A4 and hCYP2E1 as well as rCYP1A2 and rCYP2B1 were less efficient enzymes (Engst et al., 1999; Glatt et al., 1994). In incubations of 1-MP with human hepatic microsomes, 1-HMP and its derivatives formed by further oxidation accounted for 38–64% of all metabolites, indicating that side-chain oxidation is a major pathway of 1-MP metabolism (Engst et al., 1999). The 1-HMP was essentially inactive in standard in vitro genotoxicity tests, whereas it was mutagenic in bacteria in the presence of rat (Glatt et al., 1990; Surh et al., 1990) and human (Czich et al., 1994) liver cytosolic preparations supplemented with 3′-phosphoadenosine-5′-phosphosulfate (PAPS), the cofactor for sulfotransferases (SULTs). Strong mutagenic effects were also observed in various *Salmonella typhimurium* strains (Glatt et al., 2002) and V79 cells (Teubner et al., 2002) genetically engineered for the expression of mouse, rat and human SULT forms. The findings indicated that 1-HMP is bioactivated to 1-sulfooxymethylpyrene (1-SMP). Chemically synthesized 1-SMP initiated tumor growth at the site of subcutaneous injection in rats (Horn et al., 1996) and in a two-stage mouse model (Surh et al., 1990). Characteristic DNA adducts were found in hepatic DNA of rats that were treated with 1-HMP or 1-SMP by ^{32}P-postlabeling (Ma et al., 2002; Ma et al., 2000; Monnerjahn et al., 1993). Later, ultra performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) (Monien et al., 2008) and ^{1}H NMR spectroscopy were used to confirm the molecular structures of the most abundant adducts, N^2-((pyrene-1-yl)methyl)- 2′-deoxyguanosine (N^2-MP-dG) and N^6-((pyrene-1-yl)methyl)-2′-deoxyadenosine (N^6-MP-dA). These adducts were also detected in liver, lung and kidney of mice and rats treated with 1-MP corroborating the hypothesis of the bioactivation of 1-MP via 1-HMP to the ultimate genotoxic carcinogen 1-SMP (Bendadani et al., 2014b).

When 1-HMP and 1-SMP were administered at equimolar doses to rats 1-SMP formed approximately 15-fold higher levels of DNA adducts in the liver compared to 1-HMP (Surh et al., 1990). This observation suggests that 1-HMP may be detoxified by yet undisclosed metabolic pathways or that continuously formed 1-SMP may be more efficiently detoxified compared to a bolus injection of 1-SMP. A previous study showed that incubation of 1-HMP in the presence of human and rat hepatic microsomes yielded 1-pyrene carboxylic acid (1-PCA) as a product of side chain oxidation and, also, that 1-HMP may be hydroxylated in part at the pyrene ring by V79 cell lines expressing human or rodent CYPs (Engst et al., 1999). Here, we studied the metabolic pathways of 1-HMP in vivo. Male rats were treated with [^{14}C]1-HMP and unlabeled 1-HMP. The metabolites were isolated from urine and feces and their putative molecular structures were deduced from mass spectrometric and ^{1}H NMR spectroscopic data.

2. Materials and methods

2.1. Chemicals

[^{14}C]1-formylpyrene (specific activity 56.78 mCi/mmol) was purchased from NEN Radiochemicals (Boston, MA, USA). Hionic-Fluor™ and Solvable™ were from Canberra Packard (Dreieich, Germany) and Ultima-Flo™ AF and Count-off™ were from PerkinElmer (Rodgau-Jügesheim, Germany). HPLC-grade methanol and acetonitrile were obtained from Carl Roth GmbH (Karlsruhe, Germany). 1-PCA and 1-HMP and all other reagents (analytical grade or better) were from Sigma-Aldrich (Steinheim, Germany).

2.2. Synthesis of [^{14}C]1-HMP

A portion of 40 mg (172 μmol) [^{14}C]1-formylpyrene was dissolved in 4 ml ethanol and reduced by adding 10 mg (264 μmol) sodium borohydride as described previously (Ashby et al., 1990). The solution was stirred at room temperature for 16 h and dried under reduced pressure. The product was dissolved in 5 ml methanol and 2.5 ml water and purified by solid-phase extraction. A 500 mg Chromabond C18 column (Macherey & Nagel, Düren, Germany) was conditioned with 2 ml methanol, 2 ml water and 2 ml water/methanol (1:1). An aliquot of 500 μl of the product solution was loaded and the column was washed with 2 ml water/methanol (1:1). [^{14}C]1-HMP was eluted with 2 ml water/methanol (1:4) and the purity (>99%) was determined via HPLC-UV.

2.3. Animal treatment with 1-HMP and [^{14}C]1-HMP

Seven male Wistar rats (7–8 week old, body weight ~200 g) from Charles River Laboratories (Sulzfeld, Germany) were acclimatized for five days and then placed into metabolic cages. Five animals received an intraperitoneal dose of 19.3 mg (83 μmol) 1-HMP/kg body weight. For the treatment of two animals, the specific radioactivity was adjusted to 200 μCi in 19.3 mg 1-HMP/kg body weight by the addition of unlabeled 1-HMP. Urine and feces were collected after 24 and 48 h and stored at −80 °C. The animals were sacrificed and the dissected tissues were stored at −80 °C. The experiment was approved by the Ministry of Environment, Health and Consumer Protection (Landesamt für Umwelt, Gesundheit und Verbraucherschutz) of the state of Brandenburg under the reference number 32-44457 + 15.

2.4. Radioactivity in tissues

Tissue samples were mixed with two volumes of water and homogenized by an Ultra-Turrax homogenizer (IKA, Staufen, Germany). Aliquots of 0.1 g of homogenized tissue were mixed with 400 μl scintillation cocktail Solvable™ and incubated for 5 h at 50 °C. After addition of 500 μl 2-propanol the incubation was continued for 2 h at 50 °C. The mixture was decolorized by dropwise addition of 200 μl of 30% hydrogen peroxide. Finally, the samples were mixed with 4 ml Hionic-Fluor™. The radioactivity was measured by liquid scintillation counting in a β-counter LS 6500 (Beckman Coulter, Krefeld, Germany) with a calibration line of [^{14}C]1-HMP in the dose range from 0.25 to 2.0 μCi.

2.5. Preparation of HPLC samples

Aliquots of urinary samples were centrifuged at 5000g for 5 min and 25 μl of the supernatants were directly injected. For the extraction of fecal 1-HMP metabolites, feces samples were mixed with approximately two volumes of water and homogenized by an Ultra-Turrax homogenizer (IKA). The compounds were extracted using a matrix solid-phase dispersion (MSPD) protocol. Aliquots of 0.25 g of homogenized feces were blended with 1 g Isolute C18 resin (Biotage, Uppsala, Sweden). The mixture was transferred to an 8-ml reservoir tube with a bottom frit (Phenomenex, Aschaffenburg, Germany) that was coupled with a 100 mg C18ec solid-phase extraction cartridge (Macherey & Nagel), conditioned with 1 ml methanol and 1 ml of 20 mM sodium citrate buffer (pH 1.8). The coupled columns were washed with 5 ml of 20 mM sodium citrate buffer (pH 1.8) and the metabolites were eluted with 5 ml methanol. The radioactivity of the washing water and the eluate were checked by scintillation counting. The extraction efficiency was >90%. The eluate was dried under reduced pressure and the residue was taken up in 180 μl methanol and centrifuged for 5 min at 12,000g. A 50-μl portion of the supernatant was diluted with 100 μl methanol/water (1:1) and 50 μl were injected for HPLC analysis.