Formation of hepatic DNA adducts by methyleugenol in mouse models: drastic decrease by Sult1a1 knockout and strong increase by transgenic human SULT1A1/2

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Introduction

Alkenylbenzenes are secondary metabolites occurring in many herbs and spices (e.g. basil and pimento) (1,2) as well as in essential oils used for flavoring foods and cosmetics (3). Some alkenylbenzenes, such as methyleugenol (structural formula in Figure 1) and safrole, have shown carcinogenic activity in rat and mouse models, the liver being the principal target organ (4–8). The carcinogenicity of alkenylbenzenes is associated with the formation of DNA adducts (6,9–11). In general, 1′-hydroxylated metabolites of alkenylbenzenes were stronger carcinogens (9,12–14) and genotoxicants than the parent compounds (15,16). In addition, sulfotransferases (SULTs) are involved in the activation of alkenylbenzenes. This was best demonstrated for the in vivo situation with safrole. The SULT inhibitor pentachlorophenol and the usage of brachymorphic mice, characterized by a reduced synthesis of the SULT cofactor 3′-phosphoadenosine-5′-phosphosulfate (17), strongly decreased hepatic DNA adduct and tumor formation in rodents treated with 1′-hydroxysafrole or safrole (4,10,18,19). The role of individual SULT forms was studied in vitro using recombinant Salmonella typhimurium TA100 strains in mutagenicity studies with 1′-hydroxymethylbenzaldehyde (1′-OH-ME) and 3′-hydroxymethylisoeugenol (3′-OH-MIE) (20). Among the human enzymes, SULT1A1 was most efficient in the activation of both metabolites. Moreover, SULT1A1 is the most abundant SULT form in the human organism. It is highly expressed in the liver but also present in many extrahepatic tissues (21,22). Some activation was also observed with human SULT1A2, IC2 and IE1. The expression levels of these forms are usually much lower than that of SULT1A1 in human tissues. The SULT1A2 gene is located next to the SULT1A1 gene, as a result of recent gene duplication in primates (23). Among the mouse Sult forms studied, Sult1a1 was most effective in the activation of 1′-OH-ME and 3′-OH-MIE, although it did not reach the efficiency of its human ortholog (20). The SULT-dependent mutagenicity of 1′-OH-ME and 3′-OH-MIE was accompanied by the formation of DNA adducts in the target bacterial cells. Using mass spectrometry and nuclear magnetic resonance spectroscopy, we identified the major adduct as N6-(trans-methyleugenol-3′-yl)-2′-deoxyguanosine (N6-MIE-dG); a second adduct, formed at a nearly 50-fold lower level, was identified as N6-(trans-methyleugenol-3′-yl)-2′-deoxyadenosine (N6-MIE-dA) (20). The structural formulas of these adducts are presented in Figure 1. We devised ultra-performance liquid chromatography–tandem mass spectrometry methods with isotopic-labeled internal standards for the specific and sensitive detection of these adducts (20,24,25).

We recently reported the construction and characterization of mouse lines transgenic for the human SULT1A1–SULT1A2 gene cluster (26). In the meantime, we also constructed Sult1al knockout (ko) mice. From these lines, we bred humanized mice for these genes, lacking a functional mouse Sult1al but containing the human SULT1A1–SULT1A2 gene cluster.

The aim of this study was to explore the role of mouse and human SULT1A enzymes in the formation of hepatic DNA adducts by methyleugenol in an in vivo model. In addition to methyleugenol, we used its metabolites 1′-OH-ME and 3′-OH-MIE as well as methyleugenol, another alkenylbenzene metabolized to 3′-OH-MIE (27,28).

Materials and methods

Chemicals and enzymes

Methyleugenol, methyleugenol, tricarypyrin, micrococcal nuclease from Staphylococcus aureus, phosphodiesterase (type II) from bovine spleen and alkaline phosphatase from calf intestine were obtained from Sigma–Aldrich (Taufkirchen, Germany). Phosphodiesterase (type II) from calf spleen was purchased from Merck (Darmstadt, Germany). [15N]2′-deoxyguanosine was from Silantes (Munich, Germany). [15N]2′-deoxyguanosine was from Silantes (Munich, Germany). [15N]2′-deoxyguanosine was from Silantes (Munich, Germany). [15N]2′-deoxyguanosine was from Silantes (Munich, Germany). SULT1A1–SULT1A2 gene cluster.

Mouse lines and their maintenance

Wild-type FVB/N mice (subsequently termed wt mice) were purchased from Harlan (Borchen, Germany). The generation of transgenic FVB/N mice with multiple copies of the human SULT1A1–SULT1A2 gene cluster integrated in
chromosome 9 is described elsewhere \((26)\). The line termed tg1 in the original study was used. The construction of mSult1a1 knockout mice in the FVB/N background is described in Targeting vector construction and homologous recombination creating a defective mSult1a1 gene and Generation of mSult1a1 null mutant mice and their breeding with hSULT1A1/2 transgenic mice. The homozygous transgenic line was bred with wt mice to generate animals with a hemizygous gene status with respect to the human transgene (subsequently termed tg mice). The transgenic line tg1 was bred with ko mice to generate a line homozygous for both traits. For the present study, they were bred with ko mice to obtain animals hemizygous for the transgene and homozygous for the knockout (subsequently termed ko-tg mice).

**Fig. 1.** Biotransformation pathways of methyleugenol and methylisoeugenol addressed in this study.

Targeting vector construction and homologous recombination creating a defective mSult1a1 gene

For construction of the targeting vector, the Sult1a1-bearing bacterial artificial chromosome RP23-421P23 (RZPD, Berlin, Germany) was subcloned...
into a minimal vector derived from pACYC177 (NEB, Ipswich, MA) by Red/ET recombination in order to exchange the region of exons 2–4 of the mSult1a1 allele with minor modification. Briefly, hepatic microsomal fractions were administered at equimolar doses in the same vehicle. However, the intra-dose was 50 µl/kg body mass). The highest level of dG in the hydrolysate. To this end, we spiked small samples of the digestes (the equivalent of 24 µg of nucleic acid, omitting the butanol extraction step) with the internal standard [3H]dG (2 pmol) and subjected it to liquid chromatography–tandem mass spectrometry analysis. The total number of dN nucleotides was calculated knowing that 2′-deoxyguanosine constitutes 21% of all dN in the genomic DNA of the mouse (33).

Adduct and dG levels in the digestes were determined with an Acquity UPLC system equipped with an Acquity BEH-Phenyl column (1.7 µm, 2 × 100 mm) and connected to a Xevo TQ triple quadrupole mass spectrometer (all from Waters, Eschborn, Germany). Analytes were ionized by an electrospray source operating in the positive ion mode. The m/z transitions 428.1 → 177.2 (loss of dA) and 444.1 → 328.2 (loss of deoxyribose) were used for quantifying N⁶-MIE-dA and N⁶-MIE-dG, respectively (20). Additional m/z transitions were used as qualifiers (20). Likewise the m/z transition 268.2 → 152.1 (loss of deoxyribose) was used for quantifying dG.Qualifier transitions and settings of the mass spectrometer are given in Supplementary Table S-II, available at Carcinogenesis Online.

Determination of the limit of detection

The limit of detection (LOD) is determined by noise signals that are dependent on the source of the DNA and the sample preparation. It was defined as the adduct level giving a peak area of the quantifier that was the mean plus three times the standard deviation from 6 to 10 separate digests of hepatic DNA from untreated mice.

Results and discussion

Influence of SULT status on phase I metabolism of methyleugenol

Incubation of methyleugenol with hepatic reduced nicotinamide adenine dinucleotide phosphate-fortified microsomes from wt, ko, and ko-tg mice yielded similar levels and patterns of metabolites.
(Figure 2 and Table I). The proximate genotoxicants 1′-OH-ME and 3′-OH-MIE constituted nearly 53 and 12% of the metabolites detected in all cases. Thus, genetic manipulation of the SULT status did not affect the cytochrome P450-mediated metabolism of methyleugenol in mouse liver.

Absence of N°-MIE-dA and N²-MIE-dG in hepatic DNA of negative control animals and LOD

This study contained a total of 20 mice treated only with the vehicle tricaprylin but not with any alkenylbenzenes or their metabolites. None of these negative control animals provided any indication for the presence of N°-MIE-dA and N²-MIE-dG adduct in the hepatic DNA. However, noise signals matching the quantifier transition were present in some samples but were not accompanied by appropriate qualifier transitions. These signals were used for determining the LODs. They amounted to 10 N°-MIE-dA and 30 N²-MIE-dG adducts per 10⁶ dN for the standard method, and 0.6 N°-MIE-dA and 6 N²-MIE-dG adducts per 10⁶ dN for the enhanced method (with negligible impact of the methods used for DNA isolation). All protocols provided identical results, when the adduct levels were clearly above the LOD of the standard protocol.

Time course of the levels of the hepatic DNA adducts in mice treated with methyleugenol or 1′-OH-ME

The aim of these experiments was to determine a treatment duration appropriate for comparing adduct levels formed in different mouse lines. The experiments were conducted in tg animals. A first series of mice were killed at varying times (0.75–24 h) after the intraperitoneal administration of 1′-OH-ME. Adduct levels were fairly constant over the entire observation period (Table II). This result suggests that 1′-OH-ME was activated very rapidly and that adducts were rather resistant to DNA repair. The second experiment involved the oral administration of methyleugenol and killing of the animals 1.5–24 h later. In this experiment, adduct levels were lower at the first analysis time (1.5 h) than at the later times (3–24 h), when a plateau was reached. The delay in the adduct formation compared with the 1′-OH-ME treatment may be owed to the additional hydroxylation step required for the activation of methyleugenol. Moreover, absorption of methyleugenol, given orally, may have taken more time than that of 1′-OH-ME, applied intraperitoneally. Based on these time courses, animals were killed 6 h after the treatment in all subsequent experiments.

We show the results for both adducts, N°-MIE-dA and N²-MIE-dG, for the time course studies (Table II). In all treatment groups, the N²-MIE-dG was more abundant than N°-MIE-dA by a factor of 30–72. Similar findings were made in all other experiments. Therefore, we only present the results for N²-MIE-dG in Table III of the main paper. The corresponding results for the minor adduct, N°-MIE-dA, are given in Supplementary Table S-III, available at Carcinogenesis Online. The ratios of the N°-MIE-dG and N²-MIE-dA in the present study were similar to those previously found in S.experss strain treated with 1′-OH-ME, 31–65 (20), and human liver biopsy samples, ~60 (25).

Impact of the SULT status on the formation of hepatic DNA adducts in mice treated with methyleugenol or 1′-OH-ME

Methyleugenol at a dose 50 mg (280 µmol) per kg body mass formed 735 and 23 N²-MIE-dG adducts per 10⁶ dN in wt and ko mice, respectively (Table III). Thus, adduct formation was reduced by 97% in the absence of Sult1a1. At the next lower dose (28 µmol/kg), no adducts were detectable in ko animals, whereas 100 N²-MIE-dG adducts per 10⁶ dN were detected in the wt mice. On the contrary, human SULT1A1/2, alone (ko-tg) or together with mouse Sult1a1 (tg), enhanced the adduct formation strongly, 5- and 6-fold, respectively, above the wt level, at the 280 µmol/kg (Table III). Similar enhancements were observed at lower doses.

1′-OH-ME induced twice as many adducts as methyleugenol in wt mice, whereas only 12 N²-MIE-dG adducts per 10⁶ dN were detected in ko mice. Thus, knockout of Sult1a1 decreased the adduct formation by a factor of 120. Therefore, the impact of Sult1a1 was even stronger with 1′-OH-ME than with methyleugenol. The same was true for the enhancement by human SULT1A1/2. N²-MIE-dG adduct levels were 8- and 9-fold higher in ko-tg and tg mice, respectively, than in the wt mice. SULTs often compete with uridine 5′-diphospho-glucuronosyltransferases for the same substrates. Sulfation is normally a high-affinity, low-capacity pathway, whereas glucuronidation is characterized by low affinity, but high capacity (34). Thus, glucuronidation may be favored at bolus exposures, as is the case after direct intraperitoneal administration of the phase-I metabolite 1′-OH-ME. A high level of SULT enzyme may be particularly important in this situation to enhance the sulfation capacity.

Dose–response relationships for the formation of hepatic DNA adducts by methyleugenol in wt and humanized (ko-tg) mice

Methyleugenol was administered at four different dose levels, covering a 1000-fold range. DNA adducts were detected at the three highest dose levels in wt mice and at all four doses in ko-tg mice (Table III). Levels of N²-MIE-dG adducts were 4- to 6.5-fold higher in ko-tg mice than in wt mice at the individual dose levels. The dose–response relationship was nearly linear in wt mice but appeared somewhat supra-linear at low compared with high doses in ko-tg mice.

Anthony et al. (35) studied the biotransformation of [methoxy-14C] estragole in rats and mice as a function of the dose. This alkenylbenzene only differs from methyleugenol by the absence of one of the methoxy groups (in 3-position). The authors observed a high level of 14CO₂ exhalation, reflecting O-demethylation, at low dose levels with a continuous shift toward urinary excretion of the glucuronide of 14C-estrargole.
Table I. Lack of influence of the SULT status on hepatic microsomal metabolism of methyleugenol

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration (µM)</th>
<th>wt</th>
<th>ko</th>
<th>tg</th>
<th>ko-tg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyleugenol</td>
<td>110.1±4.4</td>
<td>124.2±12.4</td>
<td>107.8±13.7</td>
<td>111.1±17.1</td>
<td></td>
</tr>
<tr>
<td>1′-OH-ME</td>
<td>25.8±3.7</td>
<td>19.6±6.3</td>
<td>24.9±3.9</td>
<td>24.2±10.9</td>
<td></td>
</tr>
<tr>
<td>3′-OH-MIE</td>
<td>6.0±0.7</td>
<td>3.9±1.1</td>
<td>5.8±1.7</td>
<td>5.7±2.3</td>
<td></td>
</tr>
<tr>
<td>6-Hydroxymethyleugenol</td>
<td>6.1±0.9</td>
<td>5.2±1.7</td>
<td>2.8±1.7</td>
<td>5.6±2.6</td>
<td></td>
</tr>
<tr>
<td>2′:3′-Dihydroxy-2′,3′-dihydromethyleugenol</td>
<td>6.9±1.2</td>
<td>4.1±1.3</td>
<td>5.9±2.0</td>
<td>5.3±2.4</td>
<td></td>
</tr>
<tr>
<td>Eugenol + chavicol</td>
<td>2.1±0.2</td>
<td>1.7±0.3</td>
<td>1.9±0.3</td>
<td>1.8±0.5</td>
<td></td>
</tr>
<tr>
<td>Methyleneugenol-2′,3′-epoxide</td>
<td>3.5±1.1</td>
<td>2.9±1.4</td>
<td>2.8±0.4</td>
<td>2.3±0.7</td>
<td></td>
</tr>
<tr>
<td>Sum of recovered material</td>
<td>160.6±10.5</td>
<td>161.7±11.7</td>
<td>151.9±11.8</td>
<td>155.9±13.0</td>
<td></td>
</tr>
</tbody>
</table>

*Methyleugenol (200 µM) was incubated with hepatic microsomes (1 mg/ml protein) and a system generating reduced nicotinamide adenine dinucleotide phosphate at 37°C for 1 h. The metabolites were determined by high-performance liquid chromatography with ultraviolet detection, using chemically synthesized metabolite standards for the assignments and the calibration. Values are mean ± SD of four incubations, using microsomal preparation from separate animals. The structural formulas of the analytes and representative chromatograms are shown in Figures 1 and 2, respectively.

Table II. Time course of N⁶-MIE-dA and N²-MIE-dG adduct levels in hepatic DNA of tg mice treated with methyleugenol or 1′-OH-ME

<table>
<thead>
<tr>
<th>Time after treatment (h)</th>
<th>Adducts per 10⁴ dN</th>
<th>Methyleugenol</th>
<th>1′-OH-ME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N⁶-MIE-dG</td>
<td>N⁶-MIE-dA</td>
</tr>
<tr>
<td>0.75</td>
<td>n.i.</td>
<td>n.i.</td>
<td>6940±610</td>
</tr>
<tr>
<td>1.5</td>
<td>883±101</td>
<td>29±1</td>
<td>7820±1550</td>
</tr>
<tr>
<td>3</td>
<td>3980±450</td>
<td>81±12</td>
<td>9130±130</td>
</tr>
<tr>
<td>6</td>
<td>5120±410</td>
<td>115±4</td>
<td>9110±970</td>
</tr>
<tr>
<td>12</td>
<td>4400±950</td>
<td>87±37</td>
<td>6630±980</td>
</tr>
<tr>
<td>24</td>
<td>4920±360</td>
<td>93±12</td>
<td>8080±1290</td>
</tr>
</tbody>
</table>

*Methyleugenol (50 mg/kg body mass) was orally administered. An equimolar dose of 1′-OH-ME (280 µmol/kg body mass) was given intra-peritoneally. Animals were killed at varying times after treatment. DNA was enzymatically digested to nucleosides, followed by adduct analysis using isotope-dilution liquid chromatography–tandem mass spectrometry. Values are mean ± half range of two animals per group. n.i., not investigated.

Table III. Influence of the SULT1A status on the formation of the N²-MIE-dG adducts in hepatic DNA of mice treated with methyleugenol or 1′-OH-ME

<table>
<thead>
<tr>
<th>Treatment, µmol/kg (mg/kg)</th>
<th>N⁴-MIE-dG adducts per 10⁴ dN</th>
<th>wt</th>
<th>ko</th>
<th>tg</th>
<th>ko-tg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyleugenol</td>
<td>0.28 (0.05)</td>
<td>&lt;6</td>
<td>n.i.</td>
<td>n.i.</td>
<td>13±7</td>
</tr>
<tr>
<td></td>
<td>2.8 (0.5)</td>
<td>8±2</td>
<td>n.i.</td>
<td>n.i.</td>
<td>52±20</td>
</tr>
<tr>
<td></td>
<td>28 (5)</td>
<td>100±22</td>
<td>&lt;6</td>
<td>417±76</td>
<td>385±25</td>
</tr>
<tr>
<td></td>
<td>280 (50)</td>
<td>735±342</td>
<td>23±2</td>
<td>4500±180</td>
<td>3770±320</td>
</tr>
<tr>
<td>1′-OH-ME</td>
<td>280 (54.5)</td>
<td>1490±510</td>
<td>12±4</td>
<td>13±300±3000</td>
<td>12040±1300</td>
</tr>
</tbody>
</table>

*Animals were killed 6 h after the oral administration of methyleugenol or the intraperitoneal administration of 1′-OH-ME. DNA was enzymatically digested to nucleosides, followed by adduct analysis using isotope-dilution liquid chromatography–tandem mass spectrometry. Values are mean ± SD of four animals. They are only given, if at least three out of the four animals showed adducts levels > LOD. Otherwise the LOD is given (30 and 6 adducts per 10⁴ dN using the standard and enhanced method, respectively). n.i., not investigated. Levels of the minor adduct, N⁴-MIE-dA (presented in Supplementary Table S-III, available at Carcinogenesis Online), were nearly 1/50 of those of the N²-MIE-dG adduct.

1′-hydroxyestrargole at higher doses (1.3, 2.1, 5.2, 7.8 and 9.4% of the dose at 0.05, 5, 50, 500 and 1000 mg/kg in the mouse; similar values in the rat). Based on this observation, they proposed a dose-dependent metabolic shift in the cytochrome P450-mediated biotransformation pathways with predominance of demethylation (detoxification) at low doses and increase in 1′-hydroxylation (toxicification) at high doses. Moreover, conjugates of 1′-hydroxysafranal were observed in urine of rats, but not humans, both exposed to [¹⁴C]safranal at a dose of 0.6 mg/kg (36). This metabolic shift and the apparent absence (of glucuronides) of 1′-hydroxylated metabolites of alkenylbenzenes have been used as major reasons to postulate ‘that present exposure to methyl eugenol and estragole resulting from consumption of food, mainly spices and added as such, does not pose a significant cancer risk’ (37). It is difficult to sustain this concept for methyleugenol. We found in our preceding study that DNA adducts of this alkenylbenzene are abundant in human liver specimens. And the present experimental study provides no indication for a dose-dependent metabolic shift; even if it occurred, it did not manifest in decreased adduct formation, per dose unit, at low exposure levels. Of course, the situation may differ between different alkenylbenzenes. Moreover, we suspect that urinary excretion of glucuronides of 1′-hydroxylated metabolites is a problematic biomarker for estimating the extent of bioactivation of alkenylbenzenes. Many xenobiotics are preferentially excreted at low doses with a shift toward glucuronidation at higher exposures (34). Therefore, low levels of urinary glucuronides of 1′-hydroxyalkenylbenzenes could either indicate a low level of 1′-hydroxylation (first step of activation), a high level of sulfation (second step of activation) or a combination of both.

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Dose–response relationships of alkenylbenzenes were also addressed in other studies. Gupta et al. (32) investigated the formation of DNA adducts by safrole in mouse liver over a 10 000-fold dose range (0.001–10 mg per mouse) using a sensitive version of the 32P-post-labeling assay. Their dose–response curve was nearly linear, similar to the one observed in our study with methyleugenol in wt mice. Likewise, physiologically based kinetic models, developed by Al-Subeihi et al. (38), predict that formation of the reactive sulfonoxyl metabolites and DNA adducts by methyleugenol is linear from dose levels as low as common human exposures up to levels causing tumor formation in animal models.

Adduct formation by methyleugenol and 3′-OH-MIE in humanized (ko-tg) mice

3′-OH-MIE was equally mutagenic to 1′-OH-ME in Salmonella strains expressing human SULT1A1, human SULT1A2 or mouse Sult1a1 (20). 3′-OH-MIE is usually formed at lower levels from methyleugenol than its isomer 1′-OH-ME (ref. 29, Table I). However, 3′-OH-MIE is a major metabolite of methyleugenol (27,28). For these reasons, we also investigated methyleugenol and 3′-OH-MIE on formation of hepatic DNA adducts in humanized (ko-tg) mice. At a dose of 280 µmol/kg body mass, adducts were detectable when the enhanced assay was used (Table IV). Methylisoeugenol formed 30±5 N2-MIE-dG adducts per 10⁶ dN, which is nearly 1% of the level induced by an equimolar dose of methyleugenol. 3′-OH-MIE-treated animals showed 17±4 N2-MIE-dG adducts per 10⁶ dN, approximately 0.14% of the level observed with an equimolar dose of its isomer, 1′-OH-ME. These findings suggest an important role of detoxifying pathways for 3′-OH-MIE. This is supported by the report that methyleugenol is preferentially oxidized in the 3′ position, leading to cinnamic acid derivatives and, after side-chain shortening, to benzoic acid derivatives (28).

Conclusions

Various alkenylbenzenes are genotoxic hepatocarcinogens in rat and mouse models but are negative in standard in vitro genotoxicity tests, such as the Ames assay, even if S9 postmitochondrial fraction from a target tissue, rat liver, is used as an activating system (39,40). The reason is trivial, as SULTs are involved in the bioactivation. These enzymes are inactive in S9 preparations due to the lack of the cosubstrate, 3′-phosphohydroxynaphtoquinone-5′-phosphosulfate; and even if this cosubstrate is supplemented, extracellularly formed reactive sulfox conjugates may not penetrate into the target cell (41). However, hydroxyxylated metabolites of alkenylbenzenes are mutagenic in bacteria, when appropriate SULTs are expressed in the target cells (20). Somewhat surprisingly, 1′-OH-ME and 3′-OH-MIE showed comparable activities in these systems. However, 3′-OH-MIE formed ~700-fold lower adduct levels compared with 1′-OH-ME in ko-tg mice (Tables III and IV). The low activity in vivo appears to be due to efficient sequestration by oxidative metabolism, mediated by alcohol and aldehyde dehydrogenases (R. Kollock and H. Glätt, unpublished result). Again the required cofactor, oxidized nicotinamide adenine dinucleotide, is missing in standard S9 preparations. Thus, the fixation of standard activating systems to cytochrome P450 activities and the neglect of other enzymes can lead not only to false-negative results but also to false- (or excessively) positive results. The alkenylbenzenes and their metabolites provide good examples for both situations.

For these reasons, we sought to explore the role of SULT1A enzymes in the genotoxicity of methyleugenol in vivo in mouse liver, a known target organ of carcinogenicity. Impressively, knock-out of a single Sult gene, Sult1a1, abolished DNA adduct formation by methyleugenol and 1′-OH-ME by ≥97%. This result was surprising, as the mouse genome contains at least 15 transcribed Sult genes, and most of them are expressed in the liver (23,42–44) (and unpublished data from W.M. and H.G.). Replacement of mouse Sult1a1 by the human SULT1A1/2 cluster led to pronounced enhancement of the DNA adduct formation in our model. This finding is in agreement with our previous results with recombinant Salmonella strains. Human SULT1A1 activated 1′-OH-ME more efficiently than its mouse ortholog (20). Nevertheless, some precaution is required regarding the mouse model studied, as the tg mice have nearly 43 copies of the SULT1A1/2 cluster in their genome, and the hepatic level of SULT1A1 reaches 4.3% of the cytosolic protein (26). In humans, a copy number polymorphism of SULT1A1 has been detected (45). One to approximately five SULT1A1 copies had been found in 461 subjects (Caucasian-Americans and African-Americans) (45). Riches et al. (21) estimated the mean SULT1A1 level in 28 human liver specimens to 0.32% of the cytosolic protein. The extreme predominance of a single SULT form in the activation of methyleugenol, at least in mouse liver, may facilitate the development of physiologically based toxicokinetic models to estimate the impact of species differences, genetic polymorphisms and drug–drug interactions, as proposed by the group of I.Rietjens (38,46,47).

A single dose of 0.05 mg/kg (0.28 µmol/kg) methyleugenol was sufficient to form detectable levels of DNA adducts in the liver of ko-tg mice. This dose is 4-fold below the estimated mean daily intake of methyleugenol by humans from foods (3). In agreement with this efficiency in adduct formation even at low doses, we were able to clearly demonstrate the presence of N2-MIE-dG adducts, and often also N6-MIE-dA adducts, in 29 out of 30 human liver biopsy specimens studied (25). Based on the results in the mouse lines with varying SULT1A status, it is obvious to expect that individual variation in the SULT1A1 level, e.g. due to copy number polymorphism, may affect the susceptibility.

Supplementary Material

Supplementary Tables SI–SIII can be found at http://carcin.oxfordjournals.org/

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Conflict of Interest Statement: None declared.

References


| Table IV. DNA adduct formation by methylisoeugenol and 3′-OH-MIE in the liver of ko-tg mice |
|-----------------------------------|------------------|------------------|
| Treatment (µmol/kg)              | Adducts per 10⁶ dN |
|                                  | N²-MIE-dG | N⁶-MIE-dA |
| Methylisoeugenol                 | 280       | 30±5      | 0.7±0.3 |
| 3′-OH-MIE                        | 280       | 17±4      | <0.6   |

*Methylisoeugenol and 3′-OH-MIE were administered orally and intraperitoneally, respectively, as their isomers methyleugenol and 1′-OH-ME (Table III). Animals were killed 6 h after treatment. All analyses were carried out with the enhanced protocol. Values are mean ± SD of four animals. They are only given, if at least three out of the four animals showed adducts levels > LOD. Otherwise the LOD (6 N²-MIE-dG or 0.6 N⁶-MIE-dA per 10⁶ dN) is given.


18. Boberg,E.W. et al. (1987) Inhibition by pentachlorophenol of the initiating and promoting activities of 1′-hydroxy safrole for the formation of enzyme-altered foci and tumours in rat liver. Carcinogenesis, 8, 531–539.


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