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# Copper-mediated oxidative DNA damage induced by eugenol: possible involvement of *O*-demethylation

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**Abstract**

Eugenol used as a flavor has potential carcinogenicity. DNA adduct formation via 2,3-epoxidation pathway has been thought to be a major mechanism of DNA damage by carcinogenic allylbenzene analogs including eugenol. We examined whether eugenol can induce oxidative DNA damage in the presence of cytochrome P450 using [$^{32}$P]-5′-end-labeled DNA fragments obtained from human genes relevant to cancer. Eugenol induced Cu(II)-mediated DNA damage in the presence of cytochrome P450 (CYP)1A1, 1A2, 2C9, 2D6, or 2E1. CYP2D6 mediated eugenol-dependent DNA damage most efficiently. Piperidine and formamidopyrimidine-DNA glycosylase treatment induced cleavage sites mainly at T and G residues of the 5′-TG-3′ sequence, respectively. Interestingly, CYP2D6-treated eugenol strongly damaged C and G of the 5′-ACG-3′ sequence complementary to codon 273 of the *p53* gene. These results suggest that CYP2D6-treated eugenol can cause double base lesions. DNA damage was inhibited by both catalase and bathocuproine, suggesting that $H_2O_2$ and Cu(I) are involved. These results suggest that Cu(I)–hydroperoxo complex is primary reactive species causing DNA damage. Formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine was significantly increased by CYP2D6-treated eugenol in the presence of Cu(II). Time-of-flight-mass spectrometry demonstrated that CYP2D6 catalyzed *O*-demethylation of eugenol to produce hydroxychavicol, capable of causing DNA damage. Therefore, it is concluded that eugenol may express carcinogenicity through oxidative DNA damage by its metabolite.


*Keywords:* DNA damage; Eugenol; Cytochrome P450; 8-OxodG; Copper; Hydrogen peroxide

*Abbreviations:* 8-OxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine (and also known as 8-hydroxy-2′-deoxyguanosine); HPLC–ECD, an electrochemical detector coupled to a high-performance liquid chromatography; Fpg, *E. coli* formamidopyrimidine-DNA glycosylase; DTPA, diethylenetriamine-*N*,*N*,*N*′,*N*′′,*N*′′-pentaacetic acid; $O_2^-$, superoxide anion radical; $H_2O_2$, hydrogen peroxide; DMSO, dimethyl sulfoxide; $NADP^+$, β-nicotinamide adenine dinucleotide phosphate (oxidized form); CIP, calf intestine phosphatase; SOD, superoxide dismutase; CYP-cytochrome P450; TOF-MS, time-of-flight-mass spectrometry; G-6-PDH, glucose 6-phosphate dehydrogenase; G-6-P, glucose 6-phosphate

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## 1. Introduction

Eugenol (4-allyl-2-methoxy phenol) is a naturally occurring compound that has been used extensively as a flavoring agent and fragrance. Human exposure to eugenol also occurs through its use as an analgesic and from clove cigarettes [1,2]. Eugenol has anti-inflammation activity and might be a plausible lead candidate for further development of the COX-2 inhibitor [3]. In addition, eugenol has antimutagenic and anticarcinogenic potential [3–6]. Thus, eugenol is expected to act as a potential chemopreventive agent.

On the other hand, the US National Toxicology Program (NTP) showed that animals had an increased incidence of hepatocelluar carcinomas in male mice at low dose of eugenol. Eugenol is believed to have some mutagenic capacity in mice and should be evaluated for further toxicological effects [7]. Eugenol induced chromosomal aberrations in Chinese hamster ovary cells [8]. In animal studies, methyleugenol, a natural constituent of many plant essential oils, with structure similar to eugenol given orally to rats induced liver and stomach tumors in both sexs and kidney, mammary gland, and skin tumors in males [9]. DNA adduct formation via 2,3-epoxidation pathway is thought to be a major cause of DNA damage by carcinogenic allylbenzene analogs including eugenol and methyleugenol [10]. In addition to DNA adduct formation, eugenol also forms of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), a DNA lesion characteristic of oxidative damage, through oxidation mechanism [11]. However, the mechanism for oxidative DNA damage by eugenol remains to be clarified.

In this study, to clarify a mechanism other than DNA adduct formation, we have investigated whether oxidative DNA damage is induced by eugenol in the presence of cytochrome P450 (CYP) using [$^{32}$P]-5′-end-labeled DNA fragments obtained from the human *p16* and *p53* tumor suppressor genes and the c-Ha-*ras*-1 protooncogene. These genes are suitable for studying the mechanisms of chemical carcinogenesis because they are known to be targets for chemical carcinogens [12,13]. We also analyzed the formation of 8-oxodG using an electrochemical detector coupled to a high-performance liquid chromatography (HPLC–ECD). To clarify the ultimate carcinogen causing DNA damage, we utilized time-of-flight-mass spectrometry (TOF-MS) to identify the *O*-demethylated metabolite generated by the treatment with CYP.

## 2. Materials and methods

### 2.1. Materials

The restriction enzymes (*Sma*I, *Bss*HII, *Eco*RI, *Apa*I and *Sty*I) and glucose 6-phosphate dehydrogenase (G-6-PDH) were purchased from Boehringer Mannheim GmbH (Germany). The restriction enzymes (*Hind*III and *Xba*I) and $T_4$ polynucleotide kinase were obtained from New England Biolabs (Beverly, MA). [γ-$^{32}$P]ATP (222 TBq/mmol) was acquired from New England Nuclear (Boston, MA). Diethylenetriamine-*N*,*N*,*N*′,*N*′′,*N*′′-pentaacetic acid (DTPA) and bathocuproine disulfonic acid were procured from Dojin Chemical Co. (Kumamoto, Japan). Acrylamide, piperidine, dimethyl sulfoxide (DMSO), bisacrylamide, β-nicotinamide adenine dinucleotide phosphate (oxidized form) ($NADP^+$) and glucose 6-phosphate monosodium salt (G-6-P) were purchased from Wako (Osaka, Japan). CYP isozymes from human microsomes (1A1, 1A2, 2C9, 2D6 and 2E1) and CYP reductase (10.0 mg/ml protein from human microsomes) were purchased from Gentest Corporation (Woburn, MA). $CuCl_2$, ethanol, D-mannitol and sodium formate were acquired from Nacalai Tesque (Kyoto, Japan). Calf thymus DNA, calf intestine phosphatase (CIP), superoxide dismutase (SOD, 3000 units/mg from bovine erythrocytes), α-cyano-4-hydroxycinnamic acid, eugenol and catalase (45,000 units/mg from bovine liver) were obtained from Sigma Chemical Co. (St. Louis, MO). Nuclease $P_1$ (400 units/mg) was purchased from Yamasa Shoyu Co. (Chiba, Japan). *E. coli* formamidopyrimidine-DNA glycosylase (Fpg) was obtained from Trevigen Inc. (Gaithersburg, MD).

### 2.2. Preparation of [$^{32}$P]-5′-end-labeled DNA fragments

Exon-containing DNA fragments were obtained from the human *p53* [14] and *p16* [15] tumor suppressor genes and the c-Ha-*ras*-1 protooncogene [16]. DNA fragment of the *p53* tumor suppressor gene was prepared from pUC18 plasmid, ligated fragments

containing exons of the *p53* gene amplified by the polymerase chain reaction (PCR) method. The singly $^{32}$P-5′-end-labeled 443 bp fragment (*Apa*I 14179–*Eco*RI* 14621) was obtained according to the method described previously [17]. DNA fragment of the human c-Ha-*ras*-1 protooncogene was prepared from plasmid pbcNI, which carries a 6.6 kb *Bam*HI chromosomal DNA segment containing the c-Ha-*ras*-1 gene. The singly labeled 337 bp fragment (*Pst*I 2345–*Ava*I* 2681) was obtained according to the method described previously [18]. Nucleotide numbering starts with the *Bam*HI site [16]. Exon-containing DNA fragments were also obtained from the human *p16* tumor suppressor gene [15]; these fragments were subcloned into the pGEM®-T Easy Vector (Promega Corporation). The 484 bp fragment was further digested with *Bss*HII to obtain a singly labeled 156 bp fragment (*Bss*HII 9794–*Eco*RI* 9949) and a 324 bp fragment (*Eco*RI* 9466–*Bss*HII 9789). The asterisk indicates $^{32}$P-labeling.

### 2.3. Detection of DNA damage by eugenol treated with CYP in the presence of metal ion

Standard reaction mixtures (in a 1.5 ml Eppendorf microtube) containing eugenol, various concentrations of CYP isozyme and NADPH-generating system (200 μM $NADP^+$, 500 μM G-6-P, 0.07 units G-6-PDH and 500 μM $MgCl_2$) in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA were preincubated for 1 h at 37 °C. After preincubation, [$^{32}$P]-5′-end labeled DNA fragments, calf thymus DNA (20 μM/base) and 20 μM $CuCl_2$ were added to the mixtures, followed by the incubation for 1 h at 37 °C. Subsequently, the DNA was treated with 1 M piperidine for 20 min at 90 °C or 10 units of Fpg protein in the reaction buffer (10 mM HEPES–KOH (pH 7.4), 100 mM KCl, 10 mM EDTA and 0.1 mg/mL BSA) for 2 h at 37 °C. Fpg protein catalyzes the excision of 8-oxodG as well as Fapy residues [19–21]. After ethanol precipitation, the DNA fragments were electrophoresed and the autoradiogram was obtained by exposing X-ray film to the gel as described previously [22]. The preferred cleavage sites were determined by direct comparison of the positions of the oligonucleotides with those produced by the chemical reactions of the Maxam–Gilbert procedure [23] using a DNA-sequencing system (LKB 2010 Macrophor). A laser densitometer (LKB 2222 UltroScan XL) was used for the measurement of the relative amounts of oligonucleotides from the treated DNA fragments.

### 2.4. Analysis of 8-oxodG formation in calf thymus DNA by eugenol treated with CYP2D6

The quantity of 8-oxodG was measured utilizing a modification of the method described by Kasai et al. [24]. Standard reaction mixtures (in a 2.0 ml Eppendorf microtube) containing eugenol, 0.25 nM CYP2D6, 200 μM $NADP^+$, 500 μM G-6-P, 0.20 units G-6-PDH and 500 μM $MgCl_2$ in 400 μL of 4 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA were preincubated for 1 h at 37 °C. And then, calf thymus DNA (100 μM/base) and 20 μM $CuCl_2$ were added to the mixtures, followed by the incubation for 1 h at 37 °C. Following ethanol precipitation, the DNA fragments were digested into the nucleosides with nuclease $P_1$ and calf intestine phosphatase, and then analyzed by HPLC–ECD, as described previously [25].

### 2.5. TOF-MS analysis

TOF-MS analysis was performed on a Voyager B-RP (PerSeptive Biosystems, Framingham, MA) equipped with a nitrogen laser (337 nm, 3 ns pulse) to determine the molecular weight of eugenol metabolites generated by CYP2D6 treatment. Reaction mixture, containing 50 mM eugenol, 2.8 nM CYP2D6, 200 μM $NADP^+$, 2.5 mM G-6-P, 2.0 units G-6-PDH and 5 mM $MgCl_2$ in 100 μL of 10 mM sodium phosphate buffer (pH 7.8) were incubated for 1 h at 37 °C, and then air-dried on a stainless-steel probe tip. α-Cyano-4-hydroxycinnamic acid solution was added to the sample.

## 3. Results

### 3.1. Damage to [$^{32}$P]-labeled DNA fragments by eugenol treated with various CYP isozymes in the presence of metal ions

Eugenol treated with CYP induced Cu(II)-mediated DNA damage (Fig. 1). CYP2D6 mediated eugenol-induced DNA damage more efficiently than CYP 1A1, 1A2, 2C9 and 2E1 (Fig. 1A). CYP2D6-treated eugenol induced an increase of DNA damage in a

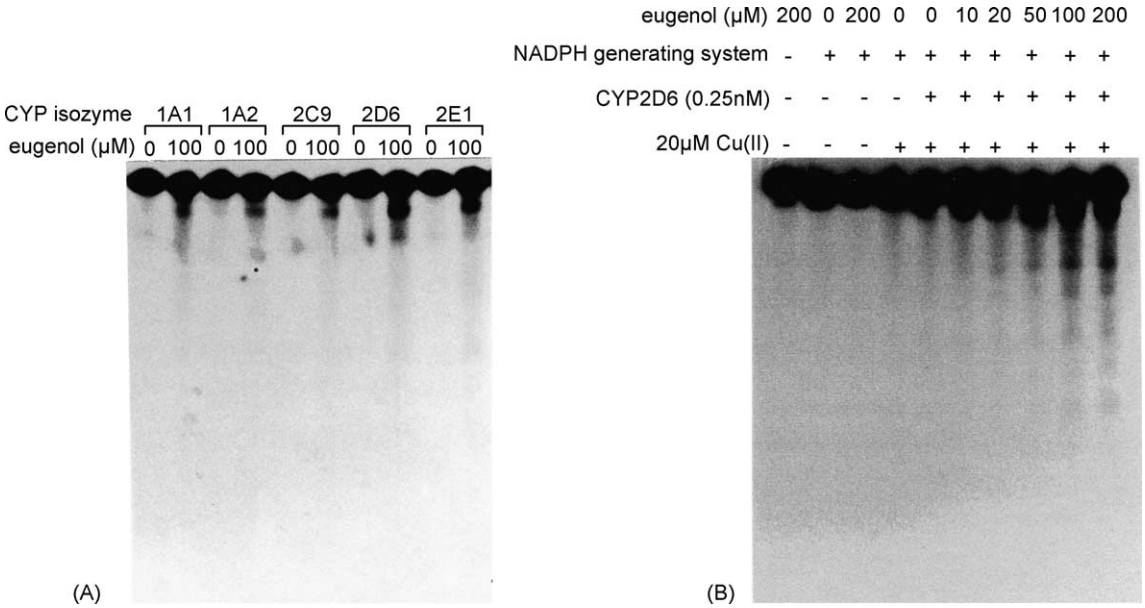


Fig. 1. Autoradiogram of $^{32}$P-labeled DNA fragments incubated with CYP-treated eugenol plus Cu(II). (A) The reaction mixtures containing indicated no or 100 μM eugenol, 0.25 nM CYP 1A1, 1A2, 2C9, 2D6 or 2E1 and NADPH-generating system (200 μM $NADP^+$, 500 μM G-6-P, 0.07 units G-6-PDH and 500 μM $MgCl_2$) in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA were preincubated for 1 h at 37 °C. After preincubation, $^{32}$P-5′-end labeled DNA fragments, calf thymus DNA (20 μM/base) and 20 μM $CuCl_2$ were added to the preincubated mixtures. (B) The reaction mixtures containing indicated concentrations of eugenol and NADPH-generating system in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA were preincubated for 1 h at 37 °C. After preincubation, $^{32}$P-5′-end labeled DNA fragments, calf thymus DNA (20 μM/base) and 20 μM $CuCl_2$ were added to the mixtures, followed by the incubation for 1 h at 37 °C. Subsequently, DNA fragments were treated with 1 M piperidine for 20 min at 90 °C, then electrophoresed on an 8% polyacrylamide/8 M urea gel. The autoradiogram was visualized by exposing an X-ray film to the gel.

dose-dependent manner (Fig. 1B). DNA damage was enhanced by piperidine treatment, suggesting that eugenol caused not only DNA strand breakage but also base modification (data not shown). Without CYP treatment, eugenol did not induce DNA damage (Fig. 1B). The treated eugenol did not induce DNA damage in the presence of other metal ions, including Co(II), Ni(II), Mn(II), Mn(III), Fe(II), Fe(III) or Fe(III)EDTA (data not shown).

### 3.2. Effects of scavengers and a metal chelator on DNA damage induced by eugenol treated with CYP2D6

Fig. 2 shows the effects of scavengers and a metal chelator on Cu(II)-mediated DNA damage induced by eugenol in the presence of CYP2D6. Catalase and bathocuproine inhibited DNA damage, suggesting the involvement of $H_2O_2$ and Cu(I). Free hydroxyl radical ($^{\bullet}$OH) scavengers, such as ethanol, mannitol, sodium formate and DMSO, showed little or no inhibitory effect on DNA damage. Methional, which is capable of scavenging both $^{\bullet}$OH and species with weaker reactivity such as Cu(I)–hydroperoxo complex [26], inhibited DNA damage. SOD showed no inhibitory effect on DNA damage.

### 3.3. Site specificity of DNA cleavage by eugenol treated with CYP2D6

The patterns of DNA cleavage induced by eugenol in the presence of Cu(II) and CYP2D6 were determined by the Maxam–Gilbert procedure [23]. An autoradiogram was obtained and scanned with a laser densitometer to measure relative intensity of DNA cleavage in the human *p53* tumor suppressor gene (Fig. 3A and B). The treated eugenol caused piperidine-labile and Fpg sensitive lesions at C and G in the 5′-ACG-3′ sequence,

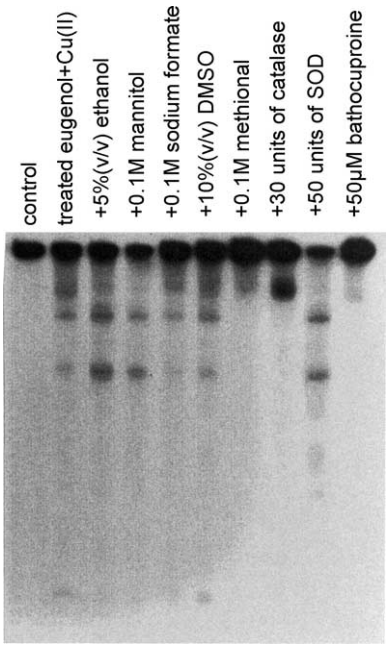


Fig. 2. Effects of scavengers and bathocuproine on DNA damage induced by eugenol treated with CYP2D6 in the presence of Cu(II). Reaction mixtures contained the $^{32}$P-5′-end-labeled 211-bp DNA fragment, 20 μM/base of calf thymus DNA, 20 μM eugenol treated with 0.25 nM CYP2D6 and 20 μM $CuCl_2$ in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. Reaction mixtures were incubated for 1 h at 37 °C. DNA fragments were treated with 1 M piperidine for 20 min at 90 °C, then electrophoresed on an 8% polyacrylamide/8 M urea gel. The autoradiogram was visualized by exposing an X-ray film to the gel.

a well-known hotspot of the *p*53 gene, respectively (Fig. 3A). With Fpg treatment, the DNA cleavage occurred mainly at guanine and cytosine residues. In addition, tandem two bases of 5′-TG-3′ site were often damaged together with Fpg and piperidine treatment (Fig. 3B). From these results, it is considered that the treated eugenol can cause double base lesions at 5′-TG-3′ and 5′-CG-3′ sequences at high frequency.

### *3.4. Formation of 8-oxodG in calf thymus DNA by eugenol treated with CYP2D6 in the presence of Cu(II)*

Using an HPLC–ECD, we measured the quantity of 8-oxodG, an indicator of oxidative base damage [24], in calf thymus DNA treated with eugenol in the presence of Cu(II) and CYP2D6. The treated eugenol induced an increase of 8-oxodG formation in a dose dependent manner (Fig. 4). Heat-inactivated CYP2D6 did not increase the eugenol-dependent 8-oxodG formation.

### *3.5. Production of O-demethyl eugenol from eugenol treated with CYP2D6*

Fig. 5 shows mass spectra of eugenol without (A) and with (B) CYP2D6 treatment. Without treatment, eugenol showed the mass spectrum with molecular ion at *m*/*e* 164 (M) (Fig. 5A). The metabolite obtained from the reaction mixture of eugenol and CYP2D6 showed the mass spectrum with molecular ion at *m*/*e* 151 (*M* + 1), which is assigned to *O*-hydroxychavicol (Fig. 5B). This result suggests that CYP2D6 catalyzes *O*-demethylation of eugenol to produce hydroxychavicol.

## 4. Discussion

Since there are a lot of CYPs in liver, we investigated the possibility that eugenol is metabolized to ultimate carcinogen causing DNA damage in the liver. The present study has demonstrated that CYP2D6 mediates eugenol-dependent DNA damage in the presence of Cu(II). When CYP1A1, CYP1A2 and CYP2E1 were added instead of CYP2D6, DNA damage was similarly observed to a lesser extent. Experiments with piperidine or Fpg treatment revealed that C and G of the 5′-ACG-3′ sequence, the complementary sequence to codon 273 (a known hotspot) in exon 8 of the *p53* gene [12,27], were significantly damaged. Eugenol treated with CYP 2D6 also formed piperidine-labile and Fpg-sensitive lesions at T and G of the 5′-TG-3′ sequence, respectively. Fpg protein mainly catalyzes the excision of piperidine-resistant 8-oxodG [19] and further oxidized piperidine-labile guanine residues [28], although Fpg also mediates cleavage of uracil glycol [29], 5-hydroxycytosine and 5,6-dihydrothymine [30] in vitro. Therefore, it is reasonably considered that the treated eugenol oxidizes the G residue of 5′-CG-3′ and 5′-TG-3′ sequences to 8-oxodG. It has been reported that reactive oxygen species induced double base lesions to DNA oligomers [31,32]. Such clustered damage, including double base lesions, appears to play an important role in carcinogenesis, because clustered damage, which can be demonstrated in living cells, is poorly repaired [33].

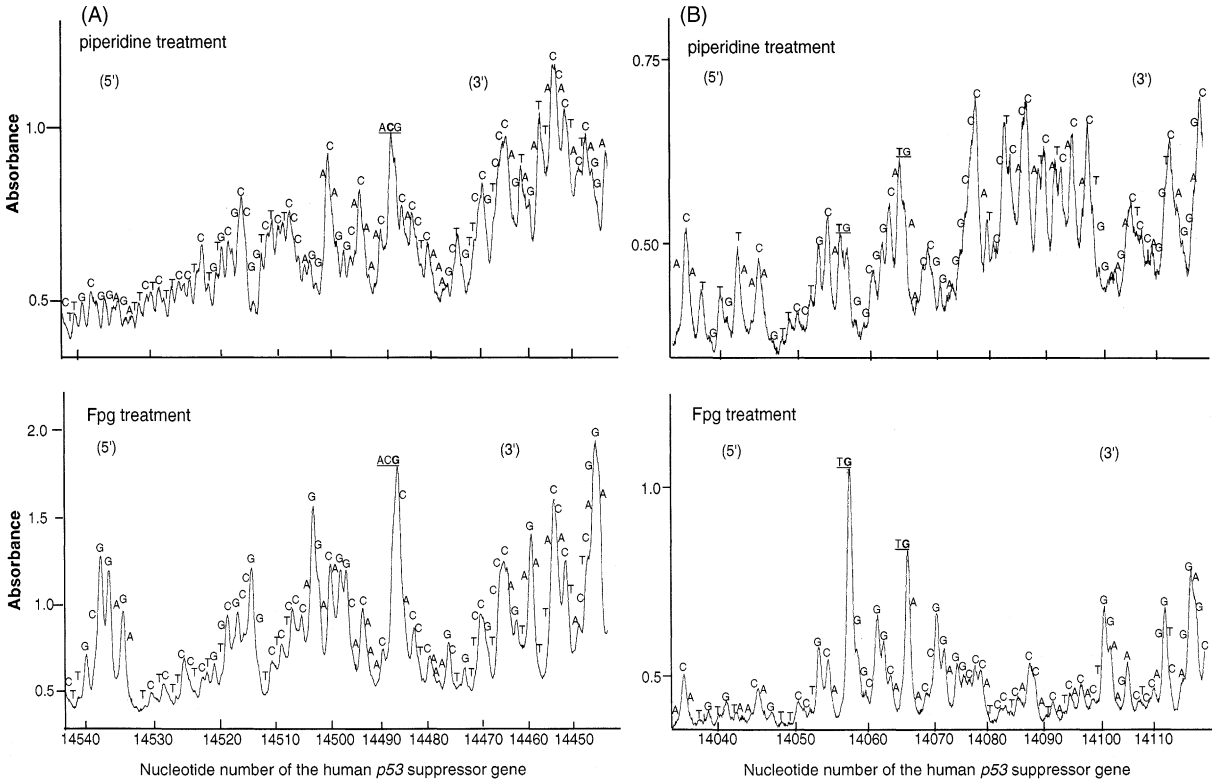


Fig. 3. Site specificity of DNA cleavage induced by eugenol treated with CYP2D6 in the presence of Cu(II). Reaction mixtures contained either the $^{32}$P-5′-end-labeled 443 bp fragment (*Apa*I 14179–*Eco*RI* 14621) (A) or the 211 bp fragment (*Hin*dIII* 13972–*Apa*I 14182) (B) derived from the *p53* tumor suppressor gene, 20 μM/base of calf thymus DNA, 200 μM eugenol treated with 0.25 nM CYP2D6 and 20 μM $CuCl_2$ in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. Reaction mixtures were incubated for 1 h at 37 °C. Following piperidine or Fpg treatment, the DNA fragments were analyzed as described in Fig. 1(legend). The relative quantities of oligonucleotides were measured by scanning the autoradiogram with a laser densitometer (LKB 2222 UltroScan XL, Pharmacia Biotech). Underlined bases represent double-base lesions detected by the treatment with piperidine and Fpg protein.

In addition, time-of-flight-mass spectrometry demonstrated that CYP2D6 catalyzed the *O*-demethylation of eugenol to produce hydroxychavicol. Hydroxychavicol appears to be the ultimate carcinogen, capable of causing DNA damage. This result is supported by reports that CYP2D6 as well as CYP 1A1, CYP1A2 and CYP2E1 can catalyze *O*-demethylation reactions [34–36].

To clarify what kind of the reactive species involved in DNA damage by eugenol treated with CYP2D6, we examined the effects of scavengers on DNA damage. The inhibitory effect of catalase suggests the involvement of $H_2O_2$. The observed protective effect of bathocuproine suggests that Cu(I) is required for DNA damage. This is supported by reports that bathocuproine inhibits the activation of $H_2O_2$ by stabilizing Cu(I) [37,38]. Typical $^{\bullet}$OH scavengers demonstrated little or no inhibitory effect, whereas methional inhibited DNA damage. This result suggests the involvement of reactive species with a similar reactivity to $^{\bullet}$OH [26]. Therefore, it is considered that reactive species such as Cu(I)–hydroperoxo complex obtained with $H_2O_2$ and Cu(I) are involved in DNA damage by CYP2D6-treated eugenol. However, $^{\bullet}$OH may participate in DNA damage through the formation of the DNA–Cu(I)–hydroperoxo complex, which releases $^{\bullet}$OH to attack the adjacent DNA constituents prior to being scavenged by $^{\bullet}$OH scavengers [39].

On the basis of these data, we propose a possible mechanism by which eugenol induces Cu(II)-mediated

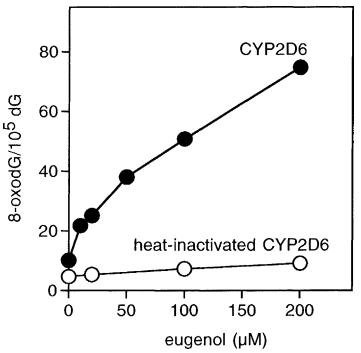


Fig. 4. Formation of 8-oxodG by eugenol treated with CYP2D6 in the presence of Cu(II). Standard reaction mixtures containing eugenol, 0.25 nM CYP2D6 and NADPH-generating system (200 μM $NADP^+$, 500 μM G-6-P, 0.07 units G-6-PDH and 500 μM $MgCl_2$) in 400 μL of 4 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA were preincubated for 2 h at 37 °C. And then, calf thymus DNA (100 μM/base) and 20 μM $CuCl_2$ were added to the mixtures. Following incubation for 1 h at 37 °C, 0.2 mM DTPA was added to stop the reaction and then the DNA was precipitated in ethanol. In certain experiments, CYP2D6 and NADPH-generating system were heated at 90 °C for 30 min for inactivation before the preincubation. The DNA was subjected to enzymatic digestion and analyzed by HPLC–ECD.

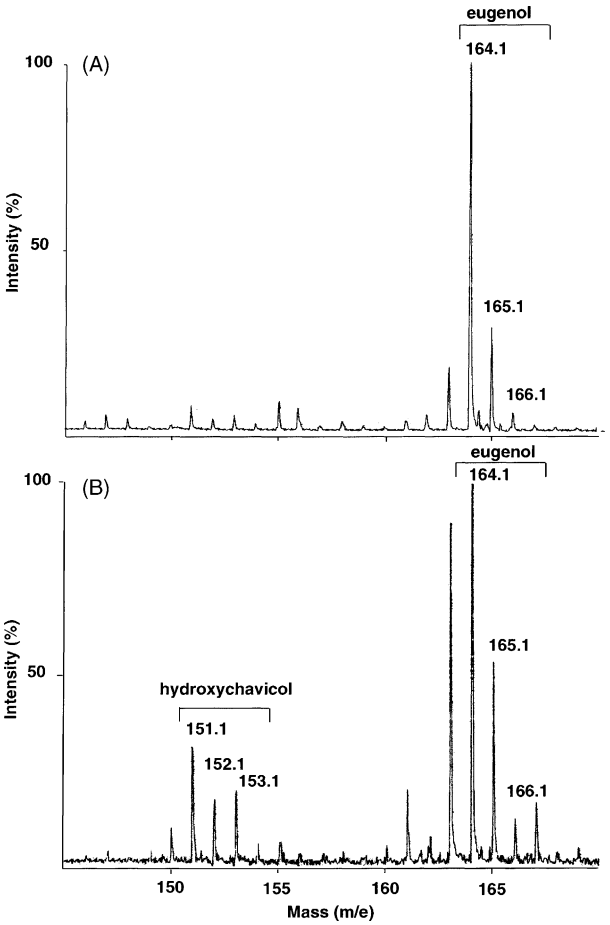


Fig. 5. Production of *O*-demethyleugenol from eugenol treated with CYP2D6. Reaction mixture, containing 50 mM eugenol, 2.8 nM CYP2D6, 200 μM $NADP^+$, 2.5 mM G-6-P, 2.0 units G-6-PDH and 5 mM $MgCl_2$ in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) were incubated for 1 h at 37 °C, and then air-dried on a stainless-steel probe tip. α-Cyano-4-hydroxycinnamic acid solution was added to the sample. TOF-MS analysis was performed on a Voyager B-RP (PerSeptive Biosystems, Framingham, MA) equipped with a nitrogen laser (337 nm, 3 ns pulse). (A) No treated eugenol; (B) CYP2D6-treated eugenol.

DNA damage (Fig. 6). Eugenol undergoes certain CYPs-catalyzed *O*-demethylation to hydroxychavicol, a catechol derivative. Hydroxychavicol is then autoxidized into the semiquinone radical, leading to the production of the corresponding *o*-quinone form. Cu(II) is reduced to Cu(I) during the autoxidation, and $O_2^-$ is concomitantly generated, followed by dismutation to $H_2O_2$. It has been also reported that hydroxychavicol produces superoxide radicals and $H_2O_2$ [40]. $H_2O_2$ interacts with Cu(I) to form the Cu(I)–hydroperoxo complex, capable of inducing DNA damage [41]. Several studies indicate that NAD(P)H may non-enzymatically reduce *o*-quinones to catechols through two-electron reduction [42]. Tissue concentrations of NAD(P)H can be as high as 100 μM [43,44]. Thus, the NADH-dependent redox cycle of hydroxychavicol may continuously generate reactive oxygen species and mediate enhancement of oxidative DNA damage.

In summary, we have demonstrated that a eugenol metabolite, hydroxychavicol, can cause oxidative DNA damage, probably double base lesions at 5′-CG-3′ and 5′-TG-3′ sequences. G residue in these sequences was oxidized to 8-oxodG, which might lead to mutation (G:C → T:A transversion) through the misreplication of DNA [45–47]. Finally, eugenol and methyleugenol may cause carcinogenesis through oxidative DNA damage in addition to DNA adduct. Further study on safety should be required when eugenol and its derivatives is used for a flavoring agent, fragrance and an analgesic.

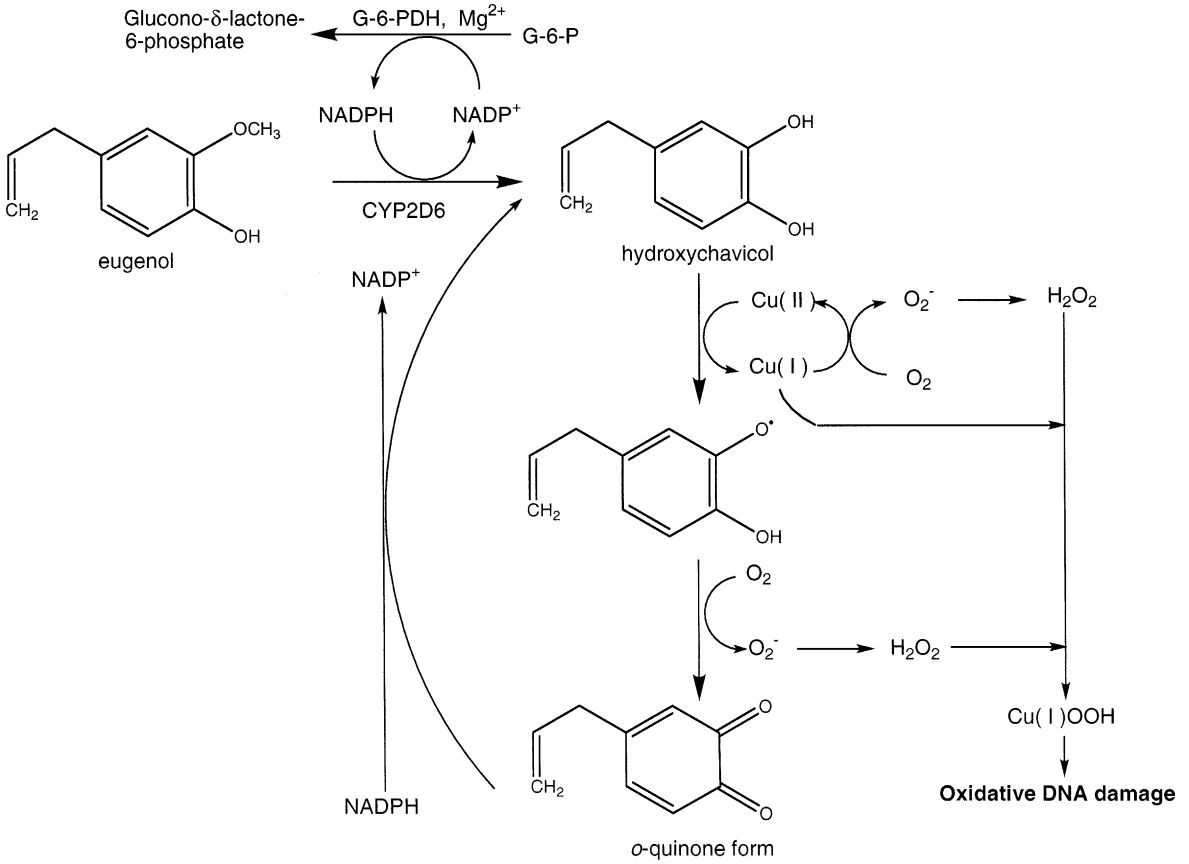


Fig. 6. A possible mechanism for Cu(II)-mediated DNA damage induced by eugenol in the presence of CYP2D6.

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