Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.5, No.6, 185-193, 2018

The Japanese Society of Toxicology

Fundamental Toxicological Sciences

URL : http://www.fundtoxicolsci.org/index_e.html

Original Article

Study of metabolic activation of the UV-filter octyl methoxycinnamate for endocrine-disrupting activity

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(Received November 28, 2018; Accepted December 6, 2018)

ABSTRACT — We examined whether octyl methoxycinnamate (OMC), a UV-filter, is metabolically activated by liver microsomes of rats and humans in respect to endocrine-disrupting action. OMC itself showed no agonistic activity towards estrogen receptor (ER) or aryl hydrocarbon receptor (AhR), and no antagonistic activity towards ER or androgen receptor (AR). The hydrolysis product, 4-methoxycinnamic acid (4-MCA), was also inactive in all the assays. In contrast, the desmethylated product, octyl hydroxycinnamate (OHC), exhibited agonistic activities towards ERα, ERβ and AhR. Importantly, when OMC was incubated with rat liver microsomes in the presence of NADPH, the major product was 4-MCA, and OHC was not formed at all. 4-MCA was also produced as the main metabolite of OMC by pooled human liver and small-intestinal microsomes. The OMC-hydrolyzing activity was higher in small-intestinal microsomes than in liver microsomes of both rats and humans, and was higher in humans than in rats. Therefore, OMC hydrolysis appears to be mainly catalyzed by small-intestinal carboxylesterase 2 isoforms, and partly by liver carboxylesterase 1 isoforms. We confirmed that OMC was hydrolyzed by human recombinant carboxylesterase isozymes, CES1b, CES1c and CES2. Our results indicate that OMC is not metabolically activated to OHC in humans, but is mainly hydrolyzed to inactive 4-MCA, suggesting that it is unlikely to pose a risk of human health in terms of endocrine-disrupting activity.

Key words: Aryl hydrogen receptor, Carboxylesterase, Estrogenic activity, *In vitro* metabolism, Octyl methoxycinnamate, UV-filter

INTRODUCTION

Octyl methoxycinnamate (3-(4-methoxyphenyl)-2-propenoic acid 2-ethylhexyl ester; OMC) has the ability to absorb and dissipate ultraviolet light, and is one of the most frequently used UV absorbants in sunscreens to protect human skin (Schlumpf *et al.,* 2004). UV filters are also employed in cosmetics, plastics, clothing, furnishings and carpets. However, OMC is highly lipophilic, and so that bioaccumulation may occur in wildlife and humans (Hagedorn-Leweke and Lippold, 1995; Hayden *et al.,* 1997; Jiang *et al.,* 1999). In addition, UV filters are often persistent in surface waters, and may pose a risk to aquatic organisms. They have been detected in fish and in the aquatic environment (e.g., in sediments and sewage sludge) (Nagtegaal *et al.,* 1997; Poiger *et al.,* 2004; Meineerling and Daniels, 2006). Indeed, OMC was reported to be toxic to marine organisms (Balmer *et al.,* 2005; Paredes *et al.,* 2014; Sieratowics *et al.,* 2011). Moreover, OMC is absorbed through human skin *in vitro* and *in vivo* (Chatelain *et al.,* 2003; Janjua *et al.,* 2004; Sarveiya *et al.,* 2004), and has been detected in human

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plasma, urine and breast milk after topical administration (Hagedorn-Leweke and Lippold, 1995; Hany and Nagel, 1995; Hayden *et al.,* 1997; Jiang *et al.,* 1999; Janjua *et al.,* 2004; Sarveiya *et al.,* 2004). Agricultural workers have been encouraged to use sunscreen to reduce their risk of skin cancer, but UV absorbers, including OMC, may increase dermal absorption of pesticides (Brand *et al.,* 2002, 2003; Pont *et al.,* 2004). In addition, some adverse reactions, such as photoallergic reactions in patients with suspected clinical photosensitivity, have been reported (Schauder and Ippen, 1997). The endocrine-disrupting properties of UV filters were summarized in a review by Krause *et al.* (2012).

Some UV absorbers show estrogenic activity in the yeast two-hybrid system with estrogen receptor and coactivators, and in reporter gene assays using various cell lines (Kawamura *et al.,* 2003; Morohoshi *et al.,* 2005; Suzuki *et al.,* 2005; Heneweer *et al.,* 2005; Watanabe *et al.,* 2015). Thus, the estrogenic activity of UV absorbers has become an important issue, as reviewed by Witorsch and Thomas (2010). In this context, there are conflicting reports concerning the estrogenic activity of OMC (Schlumpf *et al.,* 2001, 2004; Heneweer *et al.,* 2005; Wielogorska *et al.,* 2015; Gomez *et al.,* 2005; Morohoshi *et al.,* 2005; Schreurs *et al.,* 2005). Schlumpf *et al*. (2001, 2004), Heneweer *et al*. (2005) and Wielogorska *et al*. (2015) reported relatively high estrogenic activity of OMC, but other researchers reported only weak or no activity. In vivo tests have also yielded conflicting results (Schlumpf *et al.,* 2001, 2004; Klammer *et al.,* 2005; Seidlova-Wuttke *et al.,* 2006a). Schlumpf *et al.* (2001, 2004) reported an ED50 of 934 mg/kgfor estrogenic action in uterotrophic assay using immature rats, and observed increased cell proliferation of MCF-7 cells (E SCREEN), in which OMC showed similar activity to benzophenone-3. OMC, as well as other UV screens, enhanced pS2 gene transcription in MCF-7 breast cancer cells (Heneweer *et al.,* 2005). Gomez *et al.* (2005) reported that OMC exhibited relatively low estrogenic activity, but found that its activity was specific for estrogen receptor (ER)α, not ERβ. Klammer *et al.* (2005) also reported that OMC was positive in mature ovariectomized (OVX) rat assay, in accordance with Schlumph's finding in immature rats. However, Seidlova-Wuttke *et al*. (2006a) found little effect in OVX rats. OMC was also positive in vitellogenin production assay in male medaka (Inui *et al.,* 2003). Thus, the estrogenic activity of OMC remains controversial. On the other hand, Ma *et al.* (2003) reported that OMC has no antagonistic activity towards androgen receptor in the MDA-kb2 cell transcriptional-activation assay. However, OMC showed potent progesterone receptor antagonism (Schreurs *et al.,* 2005). Furthermore, the effect of OMC on thyroid function has been examined. It was reported that OMC was associated with decreases in serum TSH and thyroid hormone level in rats, and the effect of OMC were not mimicked by estradiol, supporting the idea that OMC is nonestrogenic (Klammer *et al.,* 2007; Axelstad *et al.,* 2011). OMC was also weakly positive in thyroid hormone receptor-dependent transactivation assay (Hofmann *et al.,* 2009). Janjua *et al.* (2004) examined the effects on reproductive hormone levels in human volunteers after topical application of OMC, benzophenone-3 and 3-(4-methylbenzylidene)camphor, and observed minor decreases in testosterone levels. Seidlova-Wuttke *et al.* (2006b) examined the effects of OMC on fat tissue, lipids and pituitary hormones in rats.

A possible explanation of the apparently conflicting results on the endocrine-disrupting activity of OMC in the literature would be that the compound is metabolically activated. For example, it is known that *trans*-stilbene, diphenyl and benzophenone-3 exhibit estrogenic activity after metabolic activation by the microsomal cytochrome P450 system (Kitamura *et al.,* 2008; Watanabe *et al.,* 2015). Possible metabolic pathways of OMC would include desmethylation to octyl hydroxycinnamate (3-(4-hydroxyphenyl)-2-propenoic acid 2-ethylhexyl ester; OHC) and hydrolysis to 4-methoxycinnamic acid (4-MCA), catalyzed by the cytochrome P450 system and by carboxylesterase, respectively. However, its metabolism has not so far been reported. Therefore, in this study, we examined the metabolism of OMC by liver and small-intestinal microsomes of rats and humans. We also examined the endocrine-disrupting activities of OMC and its anticipated metabolites.

MATERIALS AND METHODS

Chemicals and biochemicals

17β-Estradiol (E_2), 5α-dihydrotestosterone (DHT), OMC, 4-MCA, 4-hydroxycinnamic acid and octyl 3-hydroxy-4-methoxycinnamate (3-OH OMC) were purchased from Wako Pure Chemical Industries, Ltd. (Wako; Osaka, Japan). Bis(4-nitrophenyl)phosphate (BNPP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). OHC was synthesized according to the method of Marsh *et al.* (2003).

Pooled human liver microsomes (Lot No. 88114; 50-donor pool), small-intestinal microsomes (Lot No. 05886; 7 donor pool) and human recombinant carboxylesterase isoforms CES1b (Lot No. 36578), CES1c (Lot No. 10989), CES2 (Lot No. 20334), and Sprague Dawley rat small intestinal microsomes (Lot No. 1510219) were

obtained from BD Biosciences (San Jose, CA, USA), and XenoTech (North Hollywood, CS, USA), respectively. Dimethyl sulfoxide (DMSO; > 99.5% pure; purchased from Wako) was used as a vehicle, and all compounds used were dissolved in DMSO at a concentration of 10^{-2} M. Dulbecco's modified Eagle's medium plus Ham's F-12 nutrient mixture (D-MEM/F-12) and a penicillin-streptomycin solution (antibiotics) were obtained from GIBCO-BRL (Rockville, MD, USA). Fetal bovine serum (FBS) and charcoal-dextran-treated FBS (CD-FBS) were obtained from Hyclone (Logan, UT, USA). All compounds were diluted to the desired concentrations in appropriate medium immediately before use. The final DMSO concentration in the culture medium did not exceed 0.1%, and this concentration did not affect cell yields.

Cells and plasmids

NIH3T3 cell line derived from mouse embryo was used in the study of ER and AR. Ac2F cell line derived from rat liver was used for AhR. The cells were routinely cultured in Dulbecco's modified Eagle's medium (D-MEM) supplemented with 10% fetal bovine serum (FBS), 1% antibiotic-antimycotic mixture (Anti-Anti) and 1% MEM non-essential amino acids (MEM NEAA) at 37°C, in an atmosphere of 5% CO_2/95% air at saturating humidity, as described in our previous report. Expression plasmids, pSG5-hERα or pSG5-hERβ and a reporter plasmid, PGL3-$(ERE)_2$ were used in the study of estrogenic activity. An expression plasmid, pSG5-hAR and a reporter plasmid, pGL3-$(ARE)_2$, were used for the study of androgenic activity (Kitamura *et al*., 2003). A reporter plasmid, pGL3-XRE, was used in the study of AhR. A *Renilla* luciferase reporter, phRL-CMV (Promega), was used as the internal control.

Reporter gene assay

NIH3T3 or Ac2F cells were plated in 96-well plates (Thermo Fisher Scientific, Waltham, MA, USA) at 1 x 10^4 cells/well in D-MEM supplemented with 10% FBS, 1% Anti-Anti and 1% MEM NEAA. For estrogenic activity, NIH3T3 cells were transiently transfected with 50 ng of pSG5-hERα or pSG5-hERβ, 300 ng of pGL3-(ERE)2-luc and 3 ng of phRL-CMV with Hilymax transfection reagent (Dojindo Laboratories, Kumamoto, Japan). For the assay of androgenic activity, NIH3T3 cells were transiently transfected with 50 ng of pSG5-hAR, 300 ng of pGL3-(ARE)2-luc and 3 ng of phRL-CMV with Hilymax transfection reagent. Ac2F cells were transiently transfected with 100 ng of pGL3-XRE and 1 ng of phRL-CMV using Hilymax transfection reagent. After 24 hr, the cells were exposed to various concentrations of test compounds or 0.1% DMSO (vehicle control) in D-MEM supplemented with 1% Anti-Anti and 1% MEM NEAA (FBS was not added). After 24 hr incubation with chemicals, cells were harvested with 25 μL of passive lysis buffer (Promega). The firefly and *Renilla* luciferase activities were determined with a Dual Luciferase Assay Kit (Promega) by measuring the luminescence with a luminometer, Ascent FL (Thermo Fisher Scientific). Firefly luciferase activity was normalized to *Renilla* luciferase activity of phRL-CMV. Results are expressed as means ± standard deviation (SD) from at least three independent experiments performed in duplicate.

DMSO was used as a vehicle, and all test compounds used were dissolved in DMSO at a concentration of 3 x 10^{-2} M. The final DMSO concentration in the culture medium did not exceed 0.1%, and this concentration did not affect cell viability. All compounds were diluted to the desired concentration in the appropriate medium immediately before use.

Evaluation of agonistic and antagonistic activities

To estimate the receptor agonistic activity of the test compounds, we plotted the luminescence intensity of the assay as a dose-response curve. The antagonistic activities of the test compounds against estrogenic activity of estradiol at 1 x 10^{-6} M for ERα and ERβ, and the agonistic activity of MC (1 x 10^{-6} M) for AhR were tested. For the measurement of AR antagonistic activity, a test compound was also added to cell cultures in the presence of 1 x 10^{-6} M DHT. The androgenic activities were plotted against luminescence intensity in the respective assay.

In order to examine the effect of metabolism on the receptor agonistic and antagonistic activities of OMC, OMC (1 μmol) was incubated with 0.5 mL of rat liver microsomes (equivalent to 200 mg liver wet weight; 3 to 5 mg protein) in the presence of 10 μmol of NADPH for 30 min in a final volume of 10 mL of 0.1 M K,Na-phosphate buffer (pH 7.4). After the incubation, the mixture was extracted with 50 mL of ethyl acetate. The extract was evaporated to dryness, and the residue was dissolved in 0.1 mL of DMSO (10^{-2} M, calculated as unchanged OMC). An aliquot was used for the agonistic activity assay. The total concentration of the substrate and its metabolites was calculated from the original amount of the substrate.

Animals

Male Sprague-Dawley rats (210-230 g) were obtained from Japan SLC, Inc. (Shizuoka, Japan). The animals

were housed at 22°C and a relative humidity of 55% with a 12-hr light/dark cycle, with free access to tap water and a standard pellet diet MM-3 (Funabashi Farm, Funabashi, Japan). In some experiments, rats were given phenobarbital (PB) intraperitoneally once per day for 3 consecutive days at 80 mg/kg, or 3-methylcholanthrene (MC) or dexamethasone orally once per day for 3 consecutive days at 25 or 100 mg/kg, respectively, or acetone orally once at 3 g/kg at 24 hr before sacrifice. In other experiments, rats were given both PB intraperitoneally at 80 mg/kg and MC at 25 mg/kg orally once per day for 3 consecutive days at 24 hr before sacrifice. All experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" of Nihon Pharmaceutical University.

Rat liver preparation

Rat liver was removed and separately homogenized in four volumes of the KCl solution using a Potter-Elvehjem homogenizer. The microsomal fraction was obtained from the homogenate by successive centrifugation at 9,000 *g* for 20 min and 105,000 *g* for 60 min. The fraction was washed by resuspension in the KCl solution and resedimentation. The pellets of microsomes were resuspended in the solution to make 1 mL equivalent to 1 g of liver.

Oxidative metabolism of OMC by rat liver microsomes

An incubation mixture consisted of OMC (10 μM), 1 μmol of NADPH and 0.02 mL of liver microsomes equivalent to 20 mg liver wet weight (0.3-0.5 mg protein) in a final volume of 0.4 mL of 0.1 M K,Na-phosphate buffer (pH 7.4). The incubation was performed at 37°C for 30 min. After incubation, the mixture was extracted with 5 mL of ethyl acetate. The extract was evaporated to dryness, the residue was dissolved in 0.1 mL of methanol, and an aliquot (5 μL) was analyzed by HPLC.

Assay method of hydrolyzing activity in rat liver and small-intestinal microsomes

An incubation mixture consisted of OMC (100 μM) and 0.02 mL of liver microsomes equivalent to 20 mg liver wet weight (0.3-0.5 mg protein) or human recombinant CES isoform (CES1b, CES1c or CES2) in a final volume of 0.4 mL of 0.1 M K,Na-phosphate buffer (pH 7.4). The incubation was performed at 37°C for 10 min. After incubation, 10 μg of butylparaben was added as an internal standard and the mixture was extracted with 5 mL of ethyl acetate. The extract was evaporated to dryness, the residue was dissolved in 0.1 mL of methanol, and an aliquot (5 μL) was analyzed by HPLC.

HPLC

HPLC was performed with a Hitachi L-6000 chromatograph (Tokyo, Japan) fitted with a 125 x 4 mm Inertsil ODS-3 column (GL Science, Tokyo, Japan). The chromatograph was operated at a flow rate of 0.5 mL/min with detection at a wavelength of 254 nm. The mobile phase was acetonitrile-0.1% acetic acid (3:1, v/v). The elution times of 4-hydroxycinnamic acid, 4-MCA, butylparaben (an internal standard), OHC, 3-OH OMC and OMC were 3.4, 4.0, 11.2, 13.6, 17.3 and 22.8 min, respectively. In the hydrolytic study, the retention times of 4-MCA and butylparaben were 14.1 and 26.9 min, respectively, using the mobile phase of acetonitrile-0.1% acetic acid (1:1, v/v).

RESULTS

Agonistic activities of OMC, its metabolites, and OHC towards ERα, ERβ and AhR

The estrogenic activities of OMC, its metabolites, and OHC were examined using ERE-luciferase reporter assay in NIH3T3 cells. OMC showed no estrogenic activity against ERα and ERβ in this assay. However, the desmethylated compound, OHC, showed estrogenic activity towards both receptors, with higher activity towards ERα than towards ERβ. The compound was cytotoxic to NIH3T3 cells at concentrations above 3 x 10^{-5} M, so the apparent estrogenic activities decreased at higher concentrations. When OMC was incubated with liver microsomes of PB- and MC-cotreated rats in the presence of NADPH, the extract of the incubation mixture exhibited no estrogenic activity (Fig. 1A and 1B). OHC also exhibited agonistic activity against AhR in the range of 1 x 10^{-7} - 3 x 10^{-5} M, while OMC was inactive. OMC also showed no activity after incubation with liver microsomes of PB- and MC-cotreated rats in the presence of NADPH (Fig. 1C). The hydrolysis product, 4-MCA, did not show any agonistic activity (data not shown). Since OHC exhibited agonistic activities towards ERα, ERβ and AhR, but the extract of the incubation mixture after the metabolism did not, it appears that OMC is not converted to active metabolites such as OHC by the rat liver microsomal oxidation system.

Antagonistic activities of OMC, its metabolites, and OHC towards ERα, ERβ, AhR and AR

The antagonistic activities of OMC, its metabolites and OHC towards the estrogenic activity of 1 x 10^{-6} M estradiol at ERα and ERβ, and towards the agonistic activity of 1 x 10^{-6} M MC at AhR, as well as towards the androgenic activity of 1 x 10^{-6} M DHT at AR were examined using reporter gene assays. The tested compounds showed no

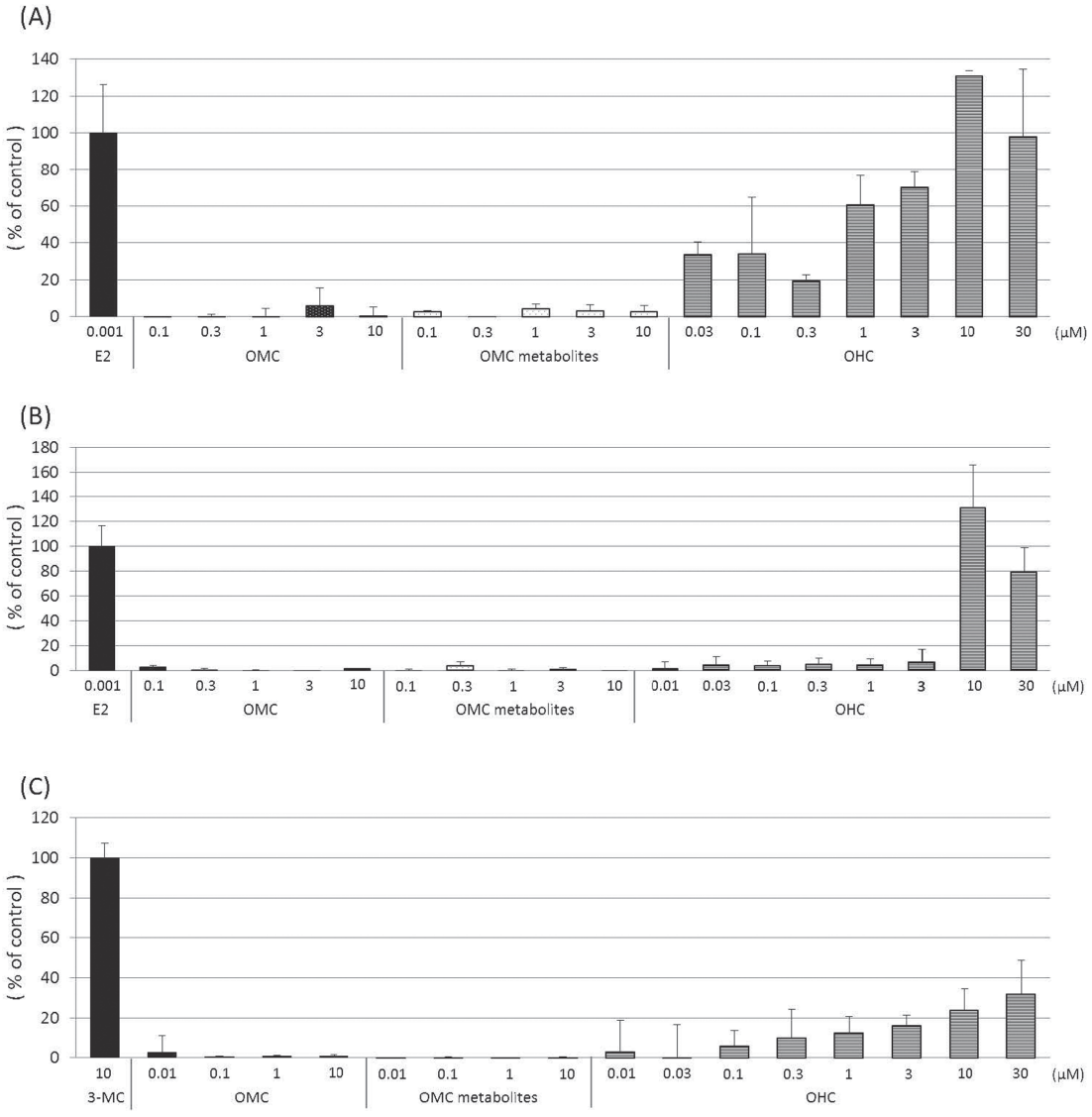

Fig. 1. Agonistic activities of OMC, its metabolites and OHC towards ERα (A), ERβ (B) and AhR (C). Agonistic activities of the test compounds towards ER and AhR were determined by luciferase reporter assay in NIH3T3 and Ac2F cells, respectively. Values represent the means ± SD of three independent experiments and are presented as percentage response, compared with the activity observed with 1 x 10^{-9} M E_2 or 1 x 10^{-5} M MC taken as 100%.

antagonistic activities in these assays in the concentration range of 1 x 10^{-7} - 1 x 10^{-5} M (data not shown).

Metabolism of OMC by liver and small-intestinal microsomes of rats and humans

Next, we examined the *in vitro* metabolism of OMC by liver and small-intestinal microsomes of rats and humans. When OMC was incubated with liver microsomes of untreated rats in the presence of NADPH, one peak was detected in the HPLC chromatogram of the extract. This peak was not detected in the control incubated without the substrate. The retention time of 4.0 min corresponded to that of authentic 4-MCA. The peak corresponding to 4-MCA was also detected when OMC was incubated with liver and small-intestinal microsomes in the absence of NADPH. The peak area after incubation

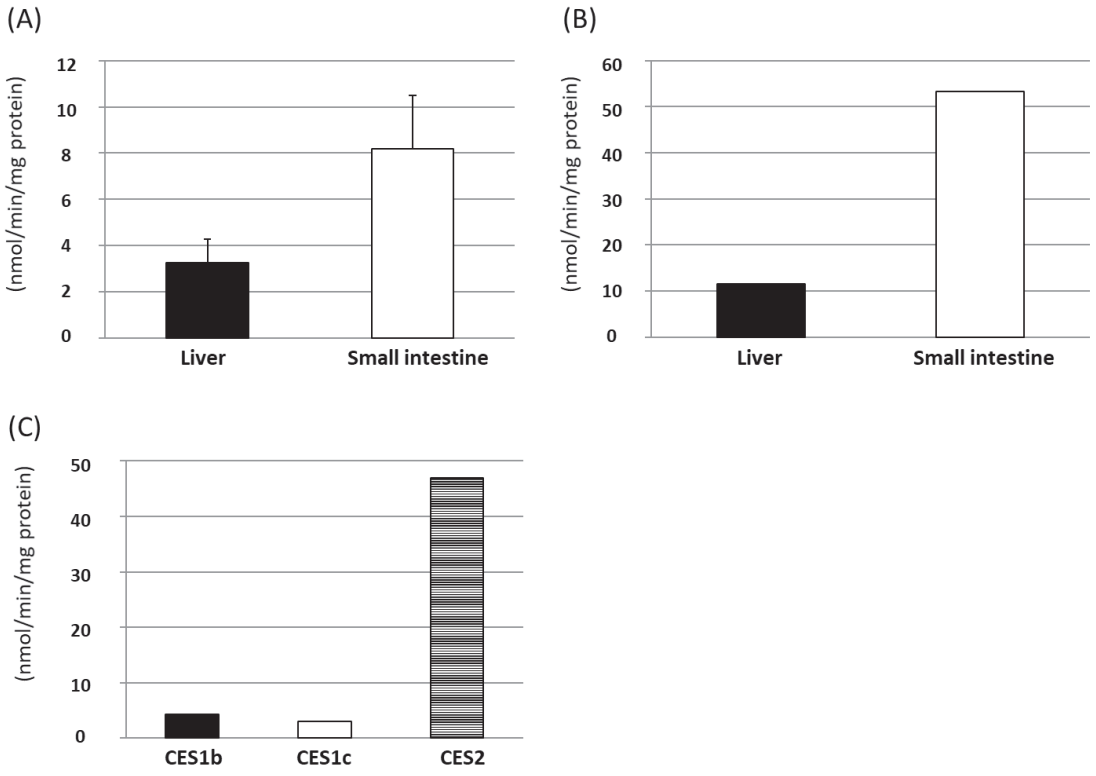

Fig. 2. Hydrolytic activity of liver and small-intestinal microsomes in rats (A) and humans (B), and human carboxylesterase isozymes (C) toward OMC. OMC was incubated with liver or small-intestinal microsomes of rats and humans at 37°C for 10 min. 4-MCA formed was determined by HPLC. Hydrolytic activity of human CES1b, CES1c and CES2 was similarly determined. Other details are described in Materials and Methods. Each bar represents the mean ± SD of four individual rats.

with small-intestinal microsomes was much higher than that with liver microsomes. Notably, the desmethylated metabolite, OHC, was not detected at the retention time of an authentic sample (13.6 min). OHC was not also detected after incubation of OMC with liver microsomes of PB-, MC-, dexamethasone- or acetone-treated rats in the presence of NADPH. Another possible metabolite of OMC is 3-hydroxylated OMC, 3-OH OMC, but no peak was detected at the retention time of an authentic sample (17.3 min). When OMC was incubated with liver and small-intestinal microsomes of humans, 4-MCA was detected as a major metabolite, while OHC and 3-OH OMC were not detected, as in the case of rats. Thus, it appears that 4-MCA is the major metabolite of OMC in rats and humans, while OHC and 3-OH OMC are not formed. In addition, the possible desmethylated metabolite of 4-MCA, 4-hydroxycinnamic acid, was not detected upon incubation of 4-MCA with rat liver microsomes.

Characterization of OMC-hydrolyzing activity in rats and humans

Interestingly, the OMC-hydrolyzing activity of small-intestinal microsomes from untreated rats was about 2.4-fold higher than that of liver microsomes from the rats (Fig. 2A). Both activities were markedly inhibited by BNPP, a specific inhibitor of carboxylesterase, at the concentration of 1 x 10^{-4} M. These results suggest that OMC is mainly hydrolyzed by carboxylesterases in rat liver and small-intestinal microsomes. Similarly, human small-intestinal microsomes showed 5-fold higher activity than human liver microsomes, though in both cases, the hydrolytic activities in humans were higher than those in rats (Fig. 2B).

We next examined the activity of the human carboxylesterase isoforms CES1b and CES1c (carboxylesterase 1 family) and CES2 (carboxylesterase 2 family) towards OMC. CES2 was most active toward OMC, and thus might be mainly responsible for the small-intestinal microsomal hydrolase activity. The liver isoforms CES1b

and CES1c both exhibited lower activities toward OMC (Fig. 2C).

DISCUSSION

In this study, we aimed to resolve the controversy as the endocrine-disrupting activity of OMC and/or its potential metabolites by examining in detail the metabolism of OMC, and the possibility of metabolic activation of OMC. We found that OMC was hydrolyzed by liver and small-intestinal microsomes of both rats and humans. The hydrolytic activity was higher in small-intestinal microsomes than in liver microsomes, and was higher in humans than in rats. Thus, the hydrolysis of OMC appears to be mainly catalyzed by small-intestinal carboxylesterase 2 isoforms, and partly by liver carboxylesterase 1 isoforms. Carboxylesterase is a serine hydrolase, and hydrolyzes substrates via a ping-pong bi-bi mechanism (Satoh and Hosokawa, 1998; Cygler *et al.,* 1993), but carboxylesterase 1 and 2 show distinct substrate specificities (Imai *et al.,* 2006; Ozaki *et al.,* 2013). For example, parabens having small alkyl side-chain substituents were hydrolyzed by carboxylesterase 1, but those having long alkyl side-chain substituents were hydrolyzed by carboxylesterase 2. Thus, it is reasonable that OMC bearing a 2-ethylhexyl (C8) side chain was actively hydrolyzed by carboxylesterase 2.

On the other hand, OMC was not converted to the *O*-desmethylated metabolite, OHC, by the P450 system. The reason for this may be the electron-withdrawing effect of the α,β-unsaturated carbonyl group attached to the phenyl ring, which causes the 4-position of the phenyl ring to be relatively electron-poor. Usually, the cytochrome P450 system favors electron-rich sites for oxidative metabolism. This may be one reason why the predominant metabolic pathway of OMC is hydrolysis by carboxylesterase (Fig. 3).

As regards the endocrine-disrupting activity of OMC, Schreurs *et al.* (2005) and Gomez *et al.* (2005) reported that OMC was negative or weakly positive in estrogen-reporter assay. Our negative result in estrogen-reporter assay using NIH3T3 cells is in agreement with their findings, as well as those of Gomez *et al.* (2005) and Seidlova-Wuttke *et al.* (2006a). However, Wielogorska *et al.* (2015) reported relatively high estrogenic activity of OMC using reporter gene assay in RGA cells, while Schlumpf *et al.* (2001, 2004) and Heneweer *et al.* (2005) reported estrogenic activity in cell proliferation and pS2 gene transcription using MCF-7 breast cancer cells. The reason for these differences is unclear, but might be related to differences in the metabolic activities of the various experimental systems used, since we found that OHC did exhibit endocrine-disrupting activities, though it was not formed in our rat and human microsomal systems.

In conclusion, our present detailed analysis of the metabolism and the estrogenic and anti-androgenic activities of OMC indicate that it is metabolized predominantly to 4-MCA, which is inactive, and not OHC, which has agonistic activities towards ERα, ERβ and AhR, in humans and rats. Thus, our findings suggest that OMC is not metabolically activated in humans, and it appears to be as safe as benzophenone-3 and 3-(4-methylbenzylidene)camphor for use in sunscreen products.

ACKNOWLEDGMENTS

This work was supported by Grants-in-Aid from the Japanese Ministry of Education, Science, Sports and Culture (KAKENHI) Grant Number 16K00559.

Conflict of interest---- The authors declare that there is no conflict of interest.

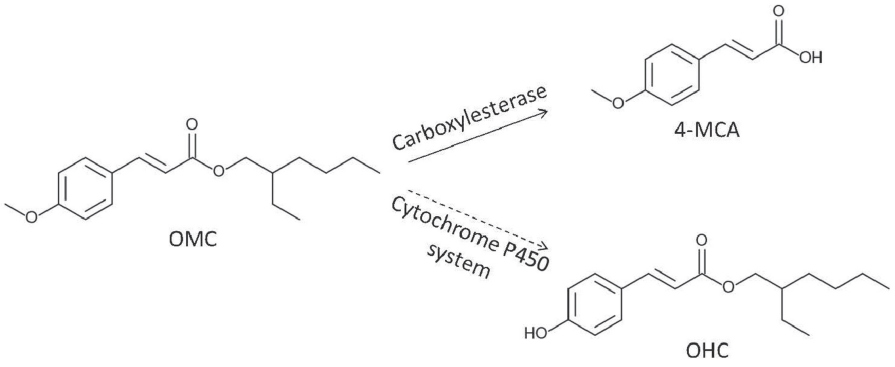

Fig. 3. Proposed metabolic pathway of OMC by liver and small-intestinal microsomal enzyme systems of rats and humans.

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