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# Omeprazole increases the efficacy of a soluble epoxide hydrolase inhibitor in a $PGE_2$ induced pain model

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ABSTRACT

Epoxyeicosatrienoic acids (EETs) are potent endogenous analgesic metabolites produced from arachidonic acid by cytochrome P450s (P450s). Metabolism of EETs by soluble epoxide hydrolase (sEH) reduces their activity, while their stabilization by sEH inhibition decreases both inflammatory and neuropathic pain. Here, we tested the complementary hypothesis that increasing the level of EETs through induction of P450s by omeprazole (OME), can influence pain related signaling by itself, and potentiate the anti-hyperalgesic effect of sEH inhibitor. Rats were treated with OME (100 mg/kg/day, p.o., 7 days), sEH inhibitor TPPU (3 mg/kg/day, p.o.) and OME (100 mg/kg/day, p.o., 7 days) + TPPU (3 mg/kg/day, p.o., last 3 days of OME dose) dissolved in vehicle PEG400, and their effect on hyperalgesia (increased sensitivity to pain) induced by $PGE_2$ was monitored. While OME treatment by itself exhibited variable effects on $PGE_2$ induced hyperalgesia, it strongly potentiated the effect of TPPU in the same assay. The significant decrease in pain with OME + TPPU treatment correlated with the increased levels of EETs in plasma and increased activities of P450 1A1 and P450 1A2 in liver microsomes. The results show that reducing catabolism of EETs with a sEH inhibitor yielded a stronger analgesic effect than increasing generation of EETs by OME, and combination of both yielded the strongest pain reducing effect under the condition of this study.



## 1. Introduction

The arachidonic acid (ARA) cascade is essential in the homeostatic regulation of physiological processes including inflammation, vasotension, and nociception (Kuehl and Egan, 1980; Davies et al., 1984; Solomon et al., 1968; Williams and Peck, 1977). Branches of this pathway include metabolites such as prostaglandins, leukotrienes and the epoxyeicosatrienoic acids (EETs). Although the existence and biological relevance of prostaglandins have been known for over half a century, the contribution of EETs to the regulation of these processes has only been realized within the past two decades (Morisseau and Hammock, 2013). Compared to the pro-inflammatory prostaglandins, EETs are potently anti-inflammatory (Thomson et al., 2012), antihypertensive (Jiang et al., 2011), anticonvulsive (Inceoglu et al., 2013) and analgesic (Inceoglu et al., 2008; Inceoglu et al., 2006). Concentrations of EETs are reported to be regulated both by their synthesis by P450s, particularly −2J2 and −2C8 in addition to others, and by their hydrolysis by sEH (Chacos et al., 1983; Imig, 2012; Morisseau and Hammock, 2013). P450's are widely known to be involved not only in the biosynthesis of chemical mediators such as steroids and epoxy-fatty acids, but also in the degradation of xenobiotics including pharmaceuticals. Here we test the hypothesis that some of the predominantly xenobiotic metabolizing P450s can contribute to the biosynthesis of biologically relevant levels of EETs.

Increasing local and systemic levels of EETs through inhibition of sEH is a well-established approach for studying the biology of these lipid mediators. Potent sEH inhibitors with sub-nanomolar potency demonstrate efficacy towards reducing pain (Inceoglu et al., 2011), hypertension (Ulu et al., 2014), fibrosis (Harris et al., 2015; Kim et al., 2015), among other biological effects (Kodani and Hammock, 2015). Interestingly, sEH inhibitors (sEHIs) are analgesic in both inflammatory (Inceoglu et al., 2006) and neuropathic pain models (Inceoglu et al., 2012), indicating that they act on a pivotal mechanism of pain perception. This mechanism occurs downstream of prostaglandin formation as evidenced by the ability of sEH inhibitors to block $PGE_2$-induced pain (Inceoglu et al., 2011).

Given that analgesic effects of sEHIs are through increasing the levels of epoxy-fatty acids including EETs, an alternative approach to increase the titer of EETs is to increase their production by inducing P450s. P450s normally associated with xenobiotic metabolism can metabolize numerous lipophilic compounds. Thus, inducers and inhibitors of these P450 enzymes can alter physiological processes by modulating the

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production of EETs. P450 induction generally occurs through activation of various nuclear receptors or the aryl hydrocarbon receptor (AhR) (Shivanna et al., 2011). Although P450 2C8 and P450 2J2 are generally considered to be the major isoforms responsible for EET production, growing evidence indicates a role in the synthesis of the oxylipins by the inducible P450 1A1 and P450 1A2 (Nebert and Karp, 2008). The potent AhR agonist 2,3,7,8-tetrachlorodibenzodioxin (TCDD) for example, leads to increase epoxy- and dihydroxy-fatty acid concentrations in mammalian liver and lung (Yang et al., 2013). Furthermore, a number of AhR-activators including TCDD and OME increased levels of various epoxy and dihydroxy metabolites in chick embryos (Diani-Moore et al., 2006). This effect was enhanced by combining TCDD with 12-(3-adamantan-1-yl-ureido)-dodecanoic acid (AUDA), a well characterized sEHI (Diani-Moore et al., 2014) suggesting the feasibility of modulating EET levels by augmenting their synthesis through inducing P450s and minimizing their metabolism by sEH inhibition.

OME is often used as an experimental probe to induce P450s (Shivanna et al., 2011; Masubuchi et al., 1997). Equally interestingly, it is a first-in-class proton pump inhibitor and one of the most commonly prescribed drugs internationally. In a number of countries OME is available as an over the counter drug and is used by millions of people with more than 720 million prescriptions issued within 15 years from the year of approval (Kaunitz, 2014; Raghunath et al., 2005; Shaheen et al., 2006). Given the roles of P450s in the synthesis of epoxy-fatty acids, their induction by OME could have functional consequences.

Therefore, in this study we asked if OME treatment would alter the physiology of pain signaling through positively modulating EET levels in a rat model. The potent sEH inhibitor TPPU was used in key experiments to slow the degradation of de novo produced EETs. To test the hypothesis that P450 induction will increase the levels of EETs and this increase in turn will functionally alter pain perception, a stringent model of pain was used. Intraplantar injection of $PGE_2$ results in intense pain. Although this pain is resistant to treatment with steroids and nonsteroidal anti-inflammatory agents (NSAIDs), earlier we demonstrated that inhibition of sEH effectively blocks this pain (Inceoglu et al., 2011). Rats were treated with OME for 7 days and tested for pain thresholds with and without TPPU. To negate the inhibitory effect of OME on certain isoforms of P450s, the effect of TPPU on pain was also studied during the OME washout period. Subsequently, P450 activity was monitored in vitro with general selective substrates; epoxygenase and hydroxylase activities were monitored in vivo using levels of EETs + dihydroxyeicosatrienoic acid (DHETs) and hydroxyeicosatetraenoic acid (HETEs) as respective biomarkers.

## 2. Materials and methods

### 2.1. Materials

OME, $PGE_2$, PEG400, methoxyresorufin, ethoxyresorufin, pentoxyresorufin, $NADP^+$, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Fisher Scientific (Houston, TX). 7-Methoxy-4-(trifluoromethyl)coumarin (MFC), 7-hydroxy-4-(trifluoromethyl)coumarin (HFC), 3-[2-(N,N-diethyl-N-methylammonium)ethyl]-7-methoxy-4-methylcoumarin (AMMC), 3-[2-(N,N-diethyl-N-methylammonium)ethyl]-7-hydroxy-4-methylcoumarin (AHMC), dibenzylfluorescein (DBF) from Corning Inc. (New York, NY); 7-benzyloxy-4-(trifluoromethyl)coumarin (BFC) from ChemBridge Corporation (San Diego, CA); 7-ethoxy-4-(trifluoromethyl)coumarin (EFC) from Molecular probes; fluorescein from Arcos Organic (New Jersey, NJ); 7-(4-methoxybenzyloxy)-4-trifluoromethylcoumarin (MOBFC) from Life Technologies (Carlsbad, CA) were used in the assay. 1-(1-propanoylpiperidin-4-yl)-3-(4-trifluoromethoxy)phenyl)urea or TPPU, 2-[$^3$H]-*trans*-1,3-diphenylpropene oxide (or [$^3$H]*t*-DPPO), [$^3$H]-*cis*-stilbene oxide (or *c*-SO), cyano(6-methoxy-2-naphthyl)methyl acetate (or CMNA) and N-(6-methoxypyridin-3-yl) octanamide (Octanoyl-MP) were synthesized in-house (Borhan et al., 1995; Shan and Hammock, 2001; Huang et al., 2007).

### 2.2. Animals and treatment

Male Sprague Dawley (SD) rats, 5–6 months old and weighing 400–500 g were used for this study. Animals were housed in a temperature and humidity controlled room and had free access to rat chow and drinking water. The study protocol was approved by the institutional animal care and use committee of University of California, Davis, and all animal experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). Animals were divided into 7 groups each containing 5–6 rats (Fig. 1). Stock solution of OME (40 mg/mL) and TPPU (10 mg/mL) were prepared in 100% PEG400 and were administered orally, the same day. The final doses of OME and TPPU were 100 mg/kg and 3 mg/kg respectively administered in a volume of 2 mL/kg of PEG400. An intermediate dose of OME was selected based on available literature on P450 1A and P450 2B induction in rats (Masubuchi et al., 1997), and the dose of TPPU was selected based on literature on the anti-hyperalgesic effect of TPPU and similar sEH inhibitors in rats (Sasso et al., 2015; Inceoglu et al., 2011; Inceoglu et al., 2012).

### 2.3. Effect of OME and TPPU on $PGE_2$ induced pain

Nociceptive withdrawal threshold was quantified with the von Frey assay using an electronic analgesiometer (IITC Inc.; Woodland hills, CA) as described earlier (Inceoglu et al., 2011; Inceoglu et al., 2012). Before the start of treatment, the response threshold of each rat was determined. Rats were then treated with vehicle (7 days), OME (7 days) and TPPU (3 days) as outlined in Fig. 1. On the days when $PGE_2$ induced pain behavior was measured, TPPU was administered 2 h before intraplantar administration of $PGE_2$. OME and vehicle were administered 1 h before injection of $PGE_2$. Although OME is an inducer of P450 1A isoforms, it is also an inhibitor of certain isoforms of P450 2C and P450 2D (Caraco et al., 1996). To reduce the effects of possible short term inhibition of P450 enzymes, in a second experiment we introduced a washout period after the last dose of OME and tested the efficacy of TPPU up to 7 days following the last OME dose. We expected that during the washout period, levels of OME would decrease below the inhibitory levels and induced P450s would still contribute to the EET pool. This experiment also provided a time course of cessation of P450 induction mediated by OME. For this second experiment, pain behavior was measured 2, 4 or 7 days after the last dose of OME. For pain behavior measurements, the withdrawal threshold was measured before $PGE_2$ and 15, 30, 45, and 60 min following $PGE_2$ administration.

### 2.4. Measurement of oxylipins in plasma

Samples were collected 2 h after treatment with $PGE_2$. For sample collection, animals were anesthetized with isoflurane, blood was collected by cardiac puncture, and livers were excised for subsequent analysis of activities of P450, esterase and hydrolase enzymes. Plasma was separated and stored at −80 °C until analysis by LC–MS/MS as described earlier (Yang et al., 2009). Epoxygenase activity was monitored using levels of EETs and their DHET metabolites in plasma. Similarly, hydroxylase activity was monitored using levels of HETEs in plasma.

### 2.5. Preparation of liver S9 and microsomal fractions

Rat liver samples were homogenized with a Polytron® bench top homogenizer (Kinematica GmbH, Luzern, Switzerland) in 4 volumes of 0.1 M Tris/HCl buffer (pH 7.4) containing 1 mM ethylenediamine tetraacetic acid (EDTA), 150 mM potassium chloride and 0.1 mM dithiothreitol (DTT) for 30 s. The homogenates were centrifuged at 10,000 g