Acetaminophen Has Lipid Composition-Dependent Membrane Interactivity That Could Be Related to Nephrotoxicity but Not to Analgesic Activity and Hepatotoxicity

Maki Mizogami a Hironori Tsuchiya b

a Department of Anesthesiology, Central Japan International Medical Center, Minokamo, Japan; b Asahi University School of Dentistry, Mizuho, Japan

Highlights of the Study

- Effects of acetaminophen and structurally related phenacetin on lipid bilayer membranes were compared by associating with their analgesic and toxic properties.
- Acetaminophen and phenacetin interacted with neuro-, hepato-, and nephro-mimetic membranes at clinically relevant concentrations to increase membrane fluidity differently.
- Lipid composition-dependent membrane interactivity of acetaminophen could be related to nephrotoxicity but not to analgesic activity and hepatotoxicity.

Keywords
Acetaminophen · Analgesic and toxic properties · Biomimetic membrane · Membrane interactivity · Phenacetin

Abstract
Objective: Although acetaminophen is one of the most widely used over-the-counter drugs, the mechanisms by which this classical drug exerts analgesic, hepatotoxic, and nephrotoxic effects remain unclear. We hypothesized that acetaminophen might act on cellular membranes of nerves, liver, and kidneys. In order to verify this hypothesis, we studied the interactivity of acetaminophen with biomimetic lipid bilayer membranes by comparing with structurally related phenacetin. Methods: Liposomal membranes (unilamellar vesicles suspended in the buffer of pH 7.4) were prepared with phospholipids and cholesterol to mimic the membrane lipid composition of neuronal cells, hepatocytes, and nephrocytes. They were subjected to reactions with acetaminophen and phenacetin at clinically relevant concentrations, followed by measuring fluorescence polarization to determine their membrane interactivity to modify membrane fluidity. Results: Acetaminophen and phenacetin interacted with neuro-mimetic and hepato-mimetic membranes to increase membrane fluidity at 10–100 μM. Both drugs were more effective in fluidizing hepato-mimetic membranes than neuro-mimetic membranes. Although the relative membrane-interacting potency was phenacetin >> acetaminophen in neuro-mimetic and hepato-mimetic membranes, such membrane effects conflicted with their relative analgesic and hepatotoxic effects. Acetaminophen and

DOI: 10.1159/000524210

Correspondence to: Hironori Tsuchiya, tsuchi-hiroki16@dent.asahi-u.ac.jp

© 2022 The Author(s). Published by S. Karger AG, Basel
This is an Open Access article licensed under the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC) (http://www.karger.com/Services/OpenAccessLicense), applicable to the online version of the article only. Usage and distribution for commercial purposes requires written permission.
phenacetin strongly interacted with nephro-mimetic membranes to increase membrane fluidity at 2–100 μM and 0.1–100 μM, respectively. Phenacetin interacted significantly with nephro-mimetic membranes at lower concentrations (<2 μM) than acetaminophen, which was consistent with their relative nephrotoxic effects. **Conclusion:** In comparison with phenacetin, lipid composition-dependent membrane interactivity of acetaminophen could be related to nephrotoxicity but not to analgesic activity and hepatotoxicity.

© 2022 The Author(s).
Published by S. Karger AG, Basel

**Materials and Methods**

**Chemicals**

Acetaminophen and phenacetin were obtained from Wako Pure Chemicals (Osaka, Japan). 1,2-Dipalmitoylphosphatidylcholine (DPPC), 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE), 1-palmitoyl-2-oleoylphosphatidylserine, and porcine brain sphingomyelin (SM) were purchased from Avanti Polar Lipids (Alabaster, AL, USA); cholesterol from Wako Pure Chemicals; and the fluorescent probe diphenyl-1,3,5-hexatriene (DPH) from Molecular Probes (Eugene, OR, USA). All other reagents were of the highest analytical grade available.

**Membrane Preparations**

Lipid bilayer membranes labeled with DPH were prepared with phospholipids and cholesterol as reported previously [6]. In brief, the dry film of phospholipids and cholesterol was dissolved with an ethanol solution of DPH. An aliquot (250 μL) of the resulting solution (total lipids of 10 mM and DPH of 50 μM) was injected four times into 199 mL of 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer of pH 7.4 containing 125 mM NaCl and 25 mM KCl under stirring above the phase transition temperatures of phospholipids. The membrane lipid composition was (1) 100 mol% DPPC for DPPC membranes that have been frequently used for membrane experiments, (2) 45 mol% POPC, 10 mol% SM, and 45 mol% cholesterol to mimic neuronal cells (neuro-mimetic membranes), (3) 35 mol% POPC, 10 mol% POPE, 5 mol% 1-palmitoyl-2-oleoylphosphatidylserine, 25 mol% SM, and 25 mol% cholesterol to mimic hepatocytes (hepato-mimetic membranes), (4) 20 mol% POPC, 20 mol% POPE, 20 mol% SM, and 40 mol% cholesterol to mimic nephrocytes (nephro-mimetic membranes), and (5) 35, 45, or 55 mol% cholesterol and phospholipids with the constant molar ratio of being POPC: SM = 45:10.

**Determination of Membrane Interactivity**

Drugs dissolved in dimethyl sulfoxide were applied to the membrane preparations so that their final concentrations were 50–200 μM for DPPC membranes, 0.5–100 μM for neuro-mimetic membranes, 10–100 μM for hepato-mimetic membranes, and 0.1–100 μM for nephro-mimetic membranes. After reacting at 37°C for 45 min, DPH fluorescence polarization was measured at 360 nm for excitation wavelength and 430 nm for emission wavelength by an FP-777 spectrofluorometer (Japan Spectroscopic Cooperation, Tokyo, Japan) equipped with a polarizer at 37°C. Polarization values were calculated by the formula \((I_{VV} - G(I_{VH})/(I_{VV} + G(I_{VH})))\), in which J is the fluorescence intensity, and the subscripts V and H refer to the vertical and horizontal orientation of excitation and emission polarizer, respectively, and G is the grating factor \((G = I_{VV}/I_{HH})\). Compared with controls, a decrease of fluorescence polarization means an increase of membrane fluidity. When comparing the interactivity between different membranes, polarization changes (%) relative to control values were used because the polarization values of intact membranes vary according to a difference of membrane lipid composition.

**Statistical Analysis**

The data were statistically analyzed by one-way ANOVA with a Bonferroni post hoc comparison using SPSS version 22 (IBM
Results

In a pilot experiment, acetaminophen and phenacetin interacted with DPPC membranes to increase membrane fluidity. The membrane effects of phenacetin relative to acetaminophen (1.00 ± 0.04) were 1.88 ± 0.11, 1.59 ± 0.05, and 2.14 ± 0.02 at 50, 100, and 200 µM, respectively.

Acetaminophen and phenacetin interacted with neuro-mimetic membranes at lower concentrations than they did with DPPC membranes (Fig. 1a). Fluorescence polarization changes (%) caused by acetaminophen and phenacetin at 100 µM for each were −3.4 ± 0.1 and −5.2 ± 0.0, respectively. The effects of phenacetin on neuro-mimetic membranes were greater than those of acetaminophen at all the tested concentrations (Fig. 1b). Compared with acetaminophen (1.00 ± 0.13), the relative potencies of phenacetin to increase neuro-mimetic membrane fluidity were 4.20 ± 0.48, 4.81 ± 0.08, 1.83 ± 0.02, and 1.53 ± 0.01 at 2, 10, 50, and 100 µM, respectively, indicating that phenacetin interacts more strongly with neuro-mimetic membranes at lower concentrations than acetaminophen does.

Acetaminophen and phenacetin interacted with hepato-mimetic membranes (Fig. 2a). The membrane effects of phenacetin relative to acetaminophen (1.00 ± 0.01) were 4.17 ± 0.11, 2.51 ± 0.03, and 1.63 ± 0.02 at 10, 50, and 100 µM, respectively. These results indicated that the interactivity of phenacetin with hepato-mimetic membranes is greater than that of acetaminophen at relatively low concentrations (Fig. 2b).

Acetaminophen and phenacetin interacted with nephro-mimetic membranes more potently than with neuro-mimetic membranes (Fig. 3a). The membrane interactivity of phenacetin was greater than that of acetaminophen and such a difference was greater with decreasing concentrations (Fig. 3b). Phenacetin showed significant membrane effects even at 0.1 and 0.2 µM. Fluorescence polarization decreases (%) relative to controls were −0.5 ± 0.0, −0.8 ± 0.1, −1.9 ± 0.1, −3.7 ± 0.1, and −6.6 ± 0.1 for 0.1, 0.5, 2, 10, and 50 µM phenacetin, respectively, and −0.3 ± 0.0, −1.0 ± 0.1, and −3.1 ± 0.1 for 2, 10, and 50 µM acetaminophen, respectively.

Fluorescence polarization values of biomimetic membranes varied depending on cholesterol content (mol%) in membranes (Fig. 4). The fluidity of intact membranes decreased with increasing membrane cholesterol.
Discussion

In the present study, acetaminophen and phenacetin have been confirmed to interact with biomimetic liposomal membranes to increase their fluidity. Kaushal et al. [7] reported that acetaminophen increases the fluidity of hepatic microsomal membranes of rats after intraperitoneal administration of acetaminophen (650 mg/kg). However, Abramson et al. [8] concluded that acetaminophen induces no changes in membrane fluidity by investigating its effects on human neutrophil and phospholipid liposomal membranes. A question arises as to why

---

Fig. 2. Effects of acetaminophen and phenacetin on hepato-mimetic membranes (a) and their relative membrane interactivity at different concentrations (b). **p < 0.01 compared with the control.

Fig. 3. Effects of acetaminophen and phenacetin on nephro-mimetic membranes (a) and their relative membrane interactivity at different concentrations (b). **p < 0.01 compared with the control.
such a difference occurred in drug-induced changes in membrane fluidity. Abramson et al. [8] measured fluorescence polarization by labeling the membranes with 1-(4-trimethylaminophenyl)-6-phenyl-1,3,5-hexatriene. 1-(4-Trimethylaminophenyl)-6-phenyl-1,3,5-hexatriene locates near the surface of phospholipid bilayers to indicate fluidity changes in the superficial hydrophilic regions of membranes, whereas DPH used by Kaushal et al. [7] and us locates in the core of phospholipid hydrocarbon chains to indicate fluidity changes in the deeper hydrophobic regions of membranes. Acetaminophen and phenacetin are considered to penetrate deep into lipid bilayers and modify the internal membrane fluidity preferentially.

Inhibition of COX-1 and COX-2 has been considered to contribute to the pharmacological and toxicological effects of typical analgesics like NSAIDs [9]. The third distinct COX isozyme COX-3 was found as a splice variant of COX-1 in the canine cerebral cortex [10]. Subsequently, acetaminophen was reported to inhibit COX-3 more potently than COX-1 and COX-2 at as low concentration as 10 and 30 μM [11]. COX-3 was also revealed to be more sensitive to phenacetin [10]. However, the clinical relevance of COX-3 is questionable because of its low expression and kinetics in humans. Apart from COX inhibition, we hypothesized that acetaminophen might interact with cellular membrane lipid bilayers at clinically relevant concentrations in association with analgesic and toxic properties.

When acetaminophen was administered to humans orally (10–20 mg/kg) or rectally (35–45 mg/kg), it achieved serum concentrations of 66–132 μM [12]. After intravenous (1 g) and oral (1–1.5 g) administration to adults, acetaminophen showed the maximal concentrations of 144–660 and 19–238 μM in plasma and 28–103 and 27–120 μM in cerebrospinal fluid, respectively [13]. Although the tested drugs increased the fluidity of neuro-mimetic membranes at 10–200 μM with the relative potency being phenacetin >> acetaminophen, such membrane effects are not consistent with the relative analgesic effects of acetaminophen and phenacetin reported so far. In the comparative evaluation of pain relief, acetaminophen and phenacetin are equivalent in analgesic efficacy [14]. The analgesic activity of phenacetin originates from its active metabolite, acetaminophen [15].

Acetaminophen and phenacetin interacted with hepa-tomimetic membranes to increase membrane fluidity at 10–100 μM. However, phenacetin showed greater membrane effects than acetaminophen, which conflicts with the reported comparative toxic effects that acetaminophen was more hepatotoxic than phenacetin [16]. Hepatotoxicity of acetaminophen may not be attributed to its membrane interaction but to hepatocyte cellular death and mitochondrial dysfunction caused by increased reactive oxygen and nitrogen species (oxidative/nitrosative stress). Overdose administered acetaminophen is metabolized to N-acetyl-p-benzoquinone imine (NAPQI) [4], which overproduces reactive oxygen species with the subsequent induction of lipid peroxidation, increasing toxic effects in the liver and other tissues [17].

Acetaminophen and phenacetin interacted with nephro-mimetic membranes at lower concentrations of 0.1–10 μM. Phenacetin was more effective in increasing nephro-mimetic membrane fluidity than acetaminophen, being consistent with their relative nephrotoxic effects [16]. Drug-induced changes in membrane fluidity were more profound when interacting with nephro-mimetic membranes than neuro-mimetic membranes. This may be due to a compositional difference in membrane cholesterol and/or phospholipids (POPE and SM). Increasing membrane cholesterol decreased the fluidity of intact membranes, which would reduce the membrane-interacting potencies of drugs.

Acetaminophen and phenacetin are metabolized to produce NAPQI which has been reported to be toxic [4]. In the literature, however, no studies have investigated whether nephrotoxic NAPQI exhibits membrane inter-

**Fig. 4.** Membrane fluidity of biomimetic membranes prepared by varying cholesterol composition. \( **p < 0.01 \) compared between different lipid compositions.
activity. Significant amounts of NAPQI metabolically produced from acetaminophen could overwhelm the cellular antioxidant system, enhancing production of reactive oxygen species. Oxidative damages are implicated in acetaminophen-induced renal injuries as well as nephrotoxic drugs [18], and apoptosis occurs in nephrocytes during acute kidney injury [19]. Reactive oxygen species increase membrane fluidity with the subsequent enhancement of membrane permeability, which is involved in cell death [20], and membrane fluidity changes are associated with apoptosis induction [5].

Drugs exhibit different activities not only by interacting directly with target proteins but also by modifying membrane lipid environments around them to alter their conformation. We used liposomal membranes consisting of lipid bilayers to focus on the drug interactions with membrane lipids not with membrane proteins. The drug actions on such protein-free membranes can significantly influence membrane protein components [21]. Drug-induced changes in membrane physicochemical properties affect membrane-embedded receptors, enzymes, and ion channels [22]. Among them, membrane fluidity is closely associated with the activity and toxicity of receptor- and enzyme-acting drugs [5, 23]. NSAIDs, local anesthetics, general anesthetics, adrenergic drugs, and opioids, which have been conventionally recognized to act on receptors and enzymes, interact with lipid membranes with the potencies correlating to pharmacological effects [5]. As with these membrane-acting drugs, the membrane interactivity of acetaminophen may be responsible for its pharmacotoxicological effects.

**Conclusion**

This is the first study to compare the membrane effects of acetaminophen and phenacetin from a mechanistic point of view. The lipid composition-dependent membrane interactivity of acetaminophen could be related to nephrotoxicity but not to analgesic activity and hepatotoxicity.

**Statement of Ethics**

Not applicable because this study includes neither human nor animal experiments.

**Conflict of Interest Statement**

The authors have no conflict of interests to declare.

**Funding Sources**

This study was supported by JSPS KAKENHI Grant No. 20K10152.

**Author Contributions**

Hironori Tsuchiya designed and conducted the study. Maki Mizogami performed the experiments and statistically analyzed the data. All the authors wrote and reviewed the manuscript.

**References**

1. Bertolini A, Ferrari A, Ottani A, Guerzoni S, Tacchi R, Leone S. Paracetamol: new vistas of an old drug. CNS Drug Rev. 2006 Jan;12(3–4): 250–75.
2. Wick EC, Grant MC, Wu CL. Postoperative multimodal analgesia pain management with nonopioid analgesics and techniques: a review. JAMA Surg. 2017 Jul;152(7):691–7.
3. Tuzuner Oncul AM, Yazicioglu D, Alanoglu Z, Demirap S, Ozturk A, Ucok C. Postoperative analgesia in impacted third molar surgery: the role of preoperative diclofenac sodium, paracetamol and lornoxicam. Med Princ Pract. 2011 Jul;20(5):470–6.
4. Ramachandran A, Jaeschke H. Acetaminophen toxicity: novel insights into mechanisms and future perspectives. Gene Expr. 2018 Mar;18(1):19–30.
5. Tsuchiya H, Mizogami M. Interaction of drugs with lipid raft membrane domains as a possible target. Drug Target Insights. 2020 Dec;14(1):34–47.
6. Tsuchiya H, Mizogami M. Discrimination of stereoisomers by their enantioselective interactions with chiral cholesterol-containing membranes. Molecules. 2017 Dec;23(1):49.
7. Kaushal R, Dave KR, Katyare SS. Paracetamol hepatotoxicity and microsomal function. Environ Toxicol Pharmacol. 1999 Mar;7(1):67–74.
8. Abramson SB, Cherksey B, Gude D, Leszynska-Pziak J, Philips MR, Blau L, et al. Nonsteroidal antiinflammatory drugs exert differential effects on neutrophil function and plasma membrane viscosity. Studies in human neutrophils and liposomes. Inflammation. 1990 Feb;14(1):11–30.
9. Aronoff DM, Oates JA, Boutaud O. New insights into the mechanism of action of acetaminophen: its clinical pharmacologic characteristics reflect its inhibition of the two prostaglandin H2 synthases. Clin Pharmacol Ther. 2006;79(1):9–19.
10. Chandrasekharan NV, Dai H, Roos KL, Evanston NK, Tomsk J, Elson TS, et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci U S A. 2002 Oct;99(21):13926–31.
11. Graham G, Scott KF. Mechanisms of action of paracetamol and related analogues. Inflammopharmacology. 2003 Dec;11(4):401–13.
12. Stocker ME, Montgomery JE. Serum paracetamol concentrations in adult volunteers following rectal administration. Br J Anaesth. 2001 Oct;87(4):638–40.
13 Langford RA, Hogg M, Bjorksten AR, Williams DL, Leslie K, Jamsen K, et al. Comparative plasma and cerebrospinal fluid pharmacokinetics of paracetamol after intravenous and oral administration. Anesth Analg. 2016 Sep;123(3):610–5.
14 Clissold SP. Paracetamol and phenacetin. Drugs. 1986 Oct;32(Suppl 4):46–59.
15 Waddington F, Naunton M, Thomas J. Paracetamol and analgesic nephropathy: are you kidneying me? Int Med Case Rep J. 2014 Dec;8:1–5.
16 Calder IC, Hart SJ, Small MC, Tange JD. Hepatotoxicity of phenacetin and paracetamol in the Gunn rat. Pathology. 1981 Oct;13(4):757–62.
17 Tripathi SS, Singh S, Garg G, Kumar R, Verma AK, Singh AK, et al. Metformin ameliorates acetaminophen-induced sub-acute toxicity via antioxidant property. Drug Chem Toxicol. 2022 Jan;45(1):52–60.
18 Abdeen A, Abdelkader A, Abdo M, Wareth G, Aboubakr M, Aleya L, et al. Protective effect of cinnamon against acetaminophen-mediated cellular damage and apoptosis in renal tissue. Environ Sci Pollut Res Int. 2019 Jan;26(1):240–9.
19 Havasi A, Borkan SC. Apoptosis and acute kidney injury. Kidney Int. 2011 Jul;80(1):29–40.
20 Tekpli X, Holme JA, Sergent O, Laganic-Gossmann D. Role for membrane remodeling in cell death: implication for health and disease. Toxicology. 2013 Feb;304:141–57.
21 Yun I, Cho ES, Jang HO, Kim UK, Choi CH, Chung IK, et al. Amphiphilic effects of local anesthetics on rotational mobility in neuronal and model membranes. Biochim Biophys Acta. 2002 Aug;1564(1):123–32.
22 Escribá PV, González-Ros JM, Goñi FM, Kin-nunen PK, Vigh L, Sánchez-Magraner L, et al. Membranes: a meeting point for lipids, proteins and therapies. J Cell Mol Med. 2008 Jun;12(3):829–75.
23 Yoshida K, Nagatoishi S, Kuroda D, Suzuki N, Murata T, Tsumoto K. Phospholipid membrane fluidity alters ligand binding activity of a G protein-coupled receptor by shifting the conformational equilibrium. Biochemistry. 2019 Feb;58(6):504–8.