High concentrations of commonly used drugs can inhibit the \textit{in vitro} glucuronidation of bisphenol A and nonylphenol in rats

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Abstract

1. 4-n-Nonylphenol and bisphenol A are endocrine disrupting chemicals that are mainly detoxified through glucuronidation. A factor that may modulate their glucuronidation rates is co-exposure to pharmaceuticals.

2. This study aimed to identify and characterize the potential metabolic interactions between 14 drugs and these two endocrine disruptors.

3. Nonylphenol and bisphenol A were co-incubated in freshly isolated rat hepatocytes with drugs at a high concentration. Statistically significant metabolic inhibition of bisphenol A and nonylphenol biotransformation was observed with nine drugs (>50% inhibition by naproxen, salicylic acid, carbamazepine and mefenamic acid). Inhibition assays of UGT activity in rat liver microsomes revealed: 1) competitive inhibition by naproxen ($K_{app} = 848.3 \, \mu M$) and carbamazepine ($K_{app} = 1023.1 \, \mu M$), 2) no inhibition by salicylic acid suggesting another mechanism of inhibition.

4. Detoxification of nonylphenol and bisphenol A was shown to be impaired by excessive concentrations of many drugs and health risk assessment should therefore address this issue.

Keywords: Bisphenol A; p-nonylphenol; drugs; interactions; hepatocytes; microsomes; metabolism; rat

Introduction

Nonylphenol (4-n-nonylphenol) and bisphenol A (4,4′-isopropylidene-2-diphenol) are widely used compounds that possess endocrine-disrupting properties. Nonylphenol is the biodegradation product of nonylphenol polyethoxylates used in many industrial and household products such as latex paints, cosmetics, and pesticides. Bisphenol A is used in the production of polycarbonate plastics and epoxy resins involved in food packaging production. Both chemicals have been detected in food (Guenther et al. 2002; Brotons et al. 1995) and drinking water (Kuch & Ballschmiter 2001).

Exposure to nonylphenol and bisphenol A produces a wide range of effects in rodents and fishes. The main concern regarding these chemicals is their endocrine disrupting properties mediated through their weak affinity for the oestrogen receptor. Their estrogenic activities were observed \textit{in vivo} with rats (Laws et al. 2000) as well as \textit{in vitro} with MCF-7 human breast cancer cells (Vivacqua et al. 2003) and human BeWo choriocarcinoma cells (Bechi et al. 2006).

Human exposure to nonylphenol and bisphenol A has been documented by many studies in which urine, blood, breast milk, and foetal serum samples were analysed (Kawaguchi et al. 2004; Otaka et al. 2003; Ikezuki et al. 2002). The study conducted by Calafat et al. (2005) on 394 adults of a reference population in the United States revealed detectable amounts of bisphenol A and nonylphenol in 95% and 51% of urine samples, respectively.

Nonylphenol and bisphenol A are extensively metabolized in the rat liver (Daidoji et al. 2003; Inoue et al.)
2001) and intestine tissues (Inoue et al. 2003), mainly to glucuronide conjugates. Glucuronidation of these compounds inhibits their ability to bind the oestrogen receptor (Matthews et al. 2001; Moffat et al. 2001) and therefore plays an important role in protecting the organism from endocrine disruption. Conjugation of these compounds to uridine diphosphate glucuronic acid (UDPGA) is catalysed by the uridine diphosphate glucuronosyltransferase 2B1 (UGT2B1) in rats (Daidoji et al. 2003; Yokota et al. 1999) and UGT2B15 (UGT2B15) in humans (Hanioka et al. 2008).

The widespread use of drugs raises the possibility of co-exposure with these hormonally active environmental contaminants. The simultaneous exposure to drugs and nonylphenol or bisphenol A can potentially lead to pharmacokinetic and pharmacodynamic interactions. Considering that these endocrine disruptor adverse effects are limited by their biotransformation to inactive glucuronidated metabolites and that they are cleared relatively rapidly, a metabolic inhibition by drugs could lead to important increases in internal levels for a given exposure. Many drugs such as naproxen, salicylic acid, ibuprofen, diclofenac are known to be glucuronidated by the UGT2B1 (King et al. 2001; Pritchard et al. 1994) in rats and are therefore potential competitive inhibitors of this conjugation reaction. Moreover, the extensive glucuronidation of drugs like acetyaminophen and valproic acid was reported to cause depletion of the cofactor UDPGA content in the liver (Howell et al. 1986; Hjelle et al. 1985), possibly leading to a reduction in glucuronidation rates. Therefore, it is important to assess the impact that drug consumption may have on bisphenol A and nonylphenol biotransformation in order to determine if drug co-exposure can potentially alter the toxic risk of these chemicals.

The first objective of this study was to identify potential in vitro metabolic interactions between bisphenol A and nonylphenol and 14 drugs that are widely used (acetaminophen, salicylic acid, ibuprofen, mefenamic acid, naproxen, amoxicillin, erythromycin, diclofenac, valproic acid, carbamazepine, gliclazide, cimetidine, ranitidine, and sulfasalazine). Concentration of these drugs at 50 times the maximum plasma concentrations ($C_{max}$) were co-incubated with bisphenol A or nonylphenol in freshly isolated rat hepatocytes. Such high drug concentrations were used in order to facilitate the identification of inhibitors in this screening part of the study. Our second aim was to characterize, using rat liver microsomes, the enzymatic inhibition of nonylphenol and bisphenol A glucuronidation by three identified drugs displaying the highest inhibitory potency. We chose to use rat material as an initial exploratory step before going to the more costly human material.

### Materials and methods

#### Chemicals

4-$n$-Nonylphenol (purity 99.9%), bisphenol A (> 99%), 4-$tert$-octylphenol (> 97%), amoxicillin (97.8%), carbamazepine (> 99%), cimetidine (> 99%), ranitidine (> 99%), gliclazide (99.5%), erythromycin (95.1%), valproic acid (100%), naproxen (98%), sulfasalazine (98%), ibuprofen (99.8%), diclofenac (> 99%), acetaminophen (100%), mefenamic acid (99.7%), salicylic acid (99.9%), UDPGA (99.7%), Type IV collagenase, William’s medium E (WME), and newborn calf serum (NCS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). High-performance liquid chromatography (HPLC) solvents were of HPLC grade (EMD Biosciences, San Diego, CA, USA). All other chemicals were of analytical grade.

#### Animals

Male Sprague–Dawley rats were purchased from Charles River Laboratories (St-Constant, Québec, Canada) with the permission of the Institutional Review Board for the protection of animals of the Université du Québec à Montréal. All animals were maintained under controlled temperature (20°C) and light cycle (12 h light/12 h darkness) with autoclaved rodent food and water available ad libitum. Rats were allowed to acclimate for 7 days prior to the experiments. Hepatocytes were isolated from rats weighing 164.9 ± 12 g (aged 42–45 days) whereas livers for microsomal preparations were obtained from rats weighing 284.5 ± 2 g (aged 63–67 days). Younger rats were used for the isolation of hepatocytes to facilitate surgical procedures.

#### Isolation of rat hepatocytes

Hepatocytes were isolated using a two-step liver perfusion method. Surgical procedures were made under 5% isoflurane anaesthesia. The portal vein was cannulated with a 22G catheter and the liver was perfused with calcium-free Hank’s balanced salt solution (HBSS) containing 25 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) and 0.5 mM ethylene glycol-bis(2-aminoethylether)-N,N,N’,N’-tetraacetic acid (EGTA) until complete removal of blood. Afterwards the liver was perfused with HBSS containing HEPES (25 mM), calcium chloride (1.7 mM) and type IV collagenase (100 U ml⁻¹) for approximately 20 min or until signs of complete digestion were observed. Perfusion solutions were adjusted to pH 7.5, continuously gassed with $O_2/CO_2$ (95%/5%) and kept at a flow rate of 15 ml min⁻¹. When perfusion was completed, the liver was extracted and dissected in WME containing HEPES.
Drugs inhibit the metabolism of bisphenol A and nonylphenol (10 mM), sodium bicarbonate (25 mM) and NCS (10%) at pH 7.5 and maintained at a temperature of 4°C. The cell suspension was filtered through a 243 µm filter. The filtrate was first washed by centrifugation at 35g for 3 min. The pellet was resuspended in WME (10% NCS) in order to proceed to a Percoll differential centrifugation at 215g for 8 min at 4°C. The remaining cells in the pellet were suspended in WME (10% NCS), and cell count and viability were assessed by Trypan blue exclusion using a haemocytometer. Only cell preparations with viability greater than 80% were kept for further experiments.

Identification of drug-pollutant interactions in isolated hepatocytes suspensions

In order to identify interactions between drugs and the two endocrine disruptors, metabolic assays were performed in hepatocyte suspensions (1 × 10⁶ cells ml⁻¹) that were incubated at 37°C in glass test tubes and agitated at 600 rpm. Suspensions were pre-incubated for 15min with or without drugs dissolved in WME (10% NCS) at concentrations 50 times higher than the maximum concentration observed in human blood (Table 1). Drug-free WME (10% NCS) was used for the controls: a no-inhibition control was incubated without any drug representing zero per cent inhibition; and another control that did not undergo any incubation was also measured to represent maximal inhibition possible. Incubations were initiated upon introduction of nonylphenol or bisphenol A at concentrations equal to reported Michaelis–Menten affinity constants (K_m), that is, 250 µM and 44 µM, respectively (Daidoji et al. 2003; Teeguarden et al. 2005). Ethanol solutions containing either nonylphenol or bisphenol A represented 0.5% of the final incubation volume for all treatments. Reactions were stopped, after 60 min for nonylphenol and 15 min for bisphenol A, using acetonitrile containing the internal standard (octylphenol) at a volume equal to that of the reaction sample. These durations of incubations are based on preliminary tests that yielded approximately 50% of pollutant disappearance. Samples were subsequently put on ice. All samples were kept at −20°C until HPLC analysis. The assays were conducted in borosilicate glassware to minimize adsorption of nonylphenol or bisphenol A. Experiments were done with hepatocytes from three rats to corroborate results. Viability was maintained in conditions where BPA and nonylphenol were administered singly. Post-exposure hepatocyte viability was also assessed with co-exposures with naproxen, salicylic acid, and carbamazepine. No appreciable difference in viability between controls and these treatments was observed. Same viability was assumed for other conditions.

Rat microsomes preparation

Rats (n = 4) were euthanized by CO₂ asphyxiation. Rat livers were removed and cut into approximately 1 g pieces prior to freezing in liquid nitrogen. Livers were kept at −80°C until microsomes preparation. A total of 2 g of liver tissue were homogenized in 8 ml of Tris (50 mM) KCl (150 mM) buffer at pH 7.5 using a tissue grinder with Teflon pestle. Samples were centrifuged

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Treatment classes</th>
<th>Plasma C_max (µM)</th>
<th>Drug concentration in assays (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>Analgesic, antipyretic</td>
<td>100^a</td>
<td>5</td>
</tr>
<tr>
<td>Salicylic acid (active metabolite of acetylsalicylic acid)</td>
<td>Non-steroidal anti-inflammatory</td>
<td>490^b</td>
<td>24.5</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Non-steroidal anti-inflammatory</td>
<td>50^c</td>
<td>2.5</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>Non-steroidal anti-inflammatory</td>
<td>29^d</td>
<td>1.45</td>
</tr>
<tr>
<td>Naproxen</td>
<td>Non-steroidal anti-inflammatory</td>
<td>374^e</td>
<td>18.7</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Non-steroidal anti-inflammatory</td>
<td>5^f</td>
<td>0.25</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>Anticonvulsive</td>
<td>12^g</td>
<td>0.6</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>Anticonvulsive</td>
<td>481^h</td>
<td>24.05</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Anticonvulsive</td>
<td>38^i</td>
<td>1.9</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>Anti-diabetic</td>
<td>46^j</td>
<td>2.3</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>H2 blocker</td>
<td>1^k</td>
<td>0.05</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>Anti-inflammatory</td>
<td>14^l</td>
<td>0.7</td>
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<td>Amoxcillin</td>
<td>Antibiotic</td>
<td>9^m</td>
<td>0.45</td>
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<tr>
<td>Erythromycin</td>
<td>Antibiotic</td>
<td>4^n</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Notes: ^Rawlins et al. (1977); ^Branemark et al. (1982); ^De Brabander et al. (2000); ^Neuvonen & Kivisto (1988); ^Mroczek et al. (1988); ^Kircheiner et al. (2003); ^Grahnen et al. (1979); ^Hussein et al. (1994); ^Miller & Ludden (1993); ^Davis et al. (2000); ^Morichau-Beauchant et al. (1986); ^Allgayer et al. (1984); ^Bodey & Nance (1972); ^Thompson et al. (1980).
for 20 min at 15 000g at 4°C. Supernatant was withdrawn and centrifuged for 60 min at 100 000g at 4°C. Supernatant containing the cytosol was discarded and microsomes were resuspended in 2 ml of Tris (50 mM) KCl (150 mM) Sucrose (250 mM) buffer at pH 7.5. Microsomes were pooled and then aliquoted into 500 µl samples before freezing in liquid nitrogen. All cell fractions were kept at −80°C until the experiments. Protein content was measured with the method described in Lowry et al. (1951).

**Drug-pollutant interaction characterization in microsome suspensions**

Characterization of metabolic inhibitions was carried out using rat liver microsome suspensions in a rotating incubator at 37°C and 600 rpm. Assays were made in Tris (50 mM) KCl (150 mM) buffer containing MgCl₂ (5 mM), polyoxyethylene 20 cetyl ether (Brij58) (0.05%) and UDPGA (5 mM). Conjugation to glucuronic acid was linear for both pollutants for 0–15 min and 0–0.5 mg of protein ml⁻¹ intervals. The protein concentration was set to 0.25 mg ml⁻¹ and the incubation was set to 15 min. Michaelis–Menten kinetic constants for both pollutants were determined by incubating microsomes with six different concentrations of substrate. In order to define the type of inhibition and the Kᵢ values for naproxen, salicylic acid, and carbamazepine, three substrate concentrations (62.5, 125 and 250 µM for nonylphenol; 50, 100 and 200 µM for bisphenol A) and four inhibitor concentrations were used. Concentrations of carbamazepine used were 125, 250, 500, and 1000 µM for bisphenol A inhibition assays and 30, 100, 300, and 1000 µM for nonylphenol inhibition assays. Concentrations of naproxen and salicylic acid were 125, 250, 500, and 1000 µM. Ethanol solutions containing the pollutants represented 1% of the final incubation volume. Inhibitors were added to the medium as aliquots solubilized in the same Tris-KCl buffer and hence no additional carrier solvent was introduced. Reactions were stopped using acetonitrile containing the internal standard (octylphenol) at a volume equal to that of the reaction sample. Samples were subsequently put on ice.

**Quantification of bisphenol A and nonylphenol metabolism**

Bisphenol A and nonylphenol metabolism rates were estimated by measuring the disappearance rate of the parent compound concentration in samples. All samples were centrifuged at 10 000g for 2 min and supernatant was injected into a Agilent 1200 series HPLC system coupled to an Agilent 1200 fluorescent detector. Bisphenol A was eluted using a Zorbax Eclipse XDB-C18 column (4.6×50 mm, 1.8 µm) and an isocratic mobile phase of acetonitrile:water (45:55 v/v) with a flow of 1.5 ml min⁻¹. The column temperature was set at 25°C. Excitation and emission wavelength were 227 nm and 313 nm, respectively. Retention times for this method were 1.4 min for bisphenol A and 16.7 min for octylphenol. In the case of nonylphenol, chromatography was performed with a Zorbax SB-C18 column (4.6×150 mm, 5 µm) and an isocratic mobile phase of acetonitrile:water (70:30 v/v) with a flow of 2 ml min⁻¹. The column temperature was set at 50°C and detection was made at 220 nm (excitation) and 315 nm (emission). Using this method, nonylphenol and octylphenol were eluted after 5.5 and 2.8 min, respectively. For both compounds, a Zorbax SB-C18 guard column (4.6×12.5 mm, 5 µm) and a 0.2 µm RRLC In-line filter (4.6 mm) preceded the chromatographic column.

**Statistics**

Bisphenol A and nonylphenol metabolism in co-incubations with pharmaceuticals was compared with that in control samples using Dunnett’s test. Statistical analyses were computed using the JMP 7.0.1 software (SAS Institute, Inc., Cary, NC, USA). Enzyme kinetic constants were determined by non-linear regression using SigmaPlot Enzyme Kinetics module (Systat Software, Inc., San Jose, CA, USA).

**Results**

**Identification of metabolic interactions**

Biotransformation of nonylphenol and bisphenol A was shown to be significantly inhibited by many drugs in freshly isolated rat hepatocytes (Figure 1). These inhibitions of metabolism displayed a similar pattern for nonylphenol and bisphenol A. The inhibitory potency and rank order of the pharmaceuticals were the same for both bisphenol A and nonylphenol. Highly significant (p < 0.001) interactions on the metabolism of the pollutants were observed with naproxen and salicylic acid with more than 85% reductions in biotransformation rates. Biotransformation of bisphenol A was also significantly inhibited by carbamazepine (p < 0.01), mefenamic acid (p < 0.01) and valproic acid (p < 0.05). In addition, carbamazepine (p < 0.001), mefenamic acid (p < 0.001), valproic acid (p < 0.001), ibuprofen (p < 0.001), diclofenac (p < 0.001), sulphasalazine (p < 0.01), and acetaminophen (p < 0.001) also significantly reduced nonylphenol biotransformation. Incubations with erythromycin, gliclazide, ranitidine and amoxicillin showed either very low inhibition or no inhibition at all. No significant changes in hepatocyte
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viability were observed when co-incubated samples were compared to drug-free control incubations (data not shown).

Bisphenol A and nonylphenol glucuronidation in rat hepatic microsomes

Bisphenol A and nonylphenol glucuronidation was characterized in permeated rat liver microsomes. Biotransformation kinetic constants were determined through the use of non-linear regression on metabolic velocity data (Figure 2). Simple one-substrate Michaelis–Menten kinetics (equation 1) best described the data with $R^2$ values above 0.96:

$$\text{Velocity} = \frac{V_{\text{max}} \times C}{K_{\text{mapp}} + C}$$

where $V_{\text{max}}$ is the maximum enzymatic velocity; $K_{\text{mapp}}$ is the affinity constant between the compound and enzyme; and $C$ is the substrate concentration (either bisphenol A or nonylphenol). Bisphenol A was glucuronidated in microsome suspensions with a $K_{\text{mapp}}$ of 114.4 ± 8.7 µM and a $V_{\text{max}}$ of 23.4 ± 0.7 nmol min$^{-1}$ mg$^{-1}$ protein. Slightly lower kinetic values were obtained for nonylphenol, which was metabolized to a glucuronide metabolite with a $K_{\text{mapp}}$ of 193.2 ± 50.0 µM and a $V_{\text{max}}$ of 19.0 ± 2.2 nmol min$^{-1}$ mg$^{-1}$ protein.

Characterization of nonylphenol and bisphenol A metabolism inhibition by naproxen, salicylic acid, and carbamazepine

Assays with rat liver microsome suspensions were carried out to evaluate the impact of co-exposure to

![Figure 1. Inhibition of nonylphenol and bisphenol A biotransformation by drugs in freshly isolated rat hepatocytes ($n = 3$). Incubations were performed at 37°C and 600 rpm for 15 and 60 min for bisphenol A (44 μM) and nonylphenol (250 μM), respectively. Initial drug concentrations were equal to 50 times the maximum plasma concentrations following a therapeutic dose in humans (see Table 1). Error bars represent the standard deviation. ***p<0.001, **p<0.01; and *p<0.05.](image)

![Figure 2. Michaelis–Menten kinetics for nonylphenol and bisphenol A in pooled rat liver microsomes ($n = 4$). Incubations were performed for 15 min at 37°C and 600 rpm using a microsomal protein concentration of 0.25 mg ml$^{-1}$. Error bars represent the standard deviation (SD).](image)
naproxen, salicylic acid, and carbamazepine on the glucuronidation of nonylphenol and bisphenol A. Concentration-dependent inhibition of nonylphenol and bisphenol A metabolism was observed with naproxen and carbamazepine. Non-linear regression analyses of the inhibitory effects of naproxen and carbamazepine, on metabolic activity data (Dixon plots presented in Figure 3) indicated competitive inhibition, which can be described by the following equation:

\[
\text{Velocity} = \frac{V_{\text{max}} \times C}{K_{\text{mapp}} \times (1 + C/K_{\text{app}}) + C}
\]

where \(V_{\text{max}}\) is the maximum enzymatic velocity; \(K_{\text{mapp}}\) is the apparent affinity constant; \(C\) is the inhibitor concentration; \(K_{\text{app}}\) is the apparent inhibition constant; and \(C\) is the substrate concentration (either bisphenol A or nonylphenol). Bisphenol A glucuronidation was inhibited by naproxen and carbamazepine with estimated \(K_{\text{app}}\) values of 848.3 ± 81.5 \(\mu\)M and 1023.1 ± 155.7 \(\mu\)M, respectively. In the case of nonylphenol, \(K_{\text{app}}\) values for naproxen and carbamazepine were, respectively, 581.7 ± 93.2 \(\mu\)M and 608.3 ± 81.3 \(\mu\)M. The regressions descriptive of competitive inhibition kinetics fitted experimental data with \(R^2 > 0.99\). No inhibition was seen in co-incubations with salicylic acid in microsome assays. The parallel slopes for the three different concentrations of either nonylphenol or bisphenol A indicate that salicylic acid does not inhibit the UDPGA-dependant biotransformation of these chemicals in microsome suspensions at concentrations between 125 and 1000 \(\mu\)M. Higher concentrations of salicylic acid showed an interaction with bisphenol A and nonylphenol metabolism but this inhibition was correlated to the lowering of pH in the buffer solution by this drug (data not shown).

**Discussion**

The growing use of drugs increases the chances of co-exposure with ubiquitous endocrine-disrupting chemicals such as nonylphenol and bisphenol A in humans. In the present study, we aimed to identify and characterize the metabolic interactions between these two environmental contaminants and 14 widely consumed drugs representing different classes of treatment. Among the studied pharmaceuticals, many significantly interfered with the biotransformation of nonylphenol and bisphenol A. This finding deserves attention in the risk assessment of these chemical contaminants, especially in the case of naproxen which was found to have a \(K_{\text{app}}\) near therapeutic blood levels.

Many metabolic interactions between drugs and pollutants were identified using freshly isolated rat hepatocytes. The rate of biotransformation of nonylphenol or bisphenol A declined when hepatocytes were pre-incubated with certain drugs for 15 min before incubation compared with controls. The fact that inhibition patterns were similar for both pollutants led the authors to hypothesize that the mechanisms of interaction are identical. Nonylphenol and bisphenol A are metabolized primarily by conjugation to glucuronic acid, a reaction primarily mediated by the enzyme UGT2B1 in rats (Daidoji et al. 2003; Yokota et al. 1999). Therefore, any interaction affecting this enzymatic reaction, cofactor synthesis, or cofactor transport would result in a similar inhibition of pollutants biotransformation. The inhibitions seen with naproxen, salicylic acid, ibuprofen, and diclofenac could be explained by the fact that all these drugs were found to be metabolized to some extent by the UGT2B1 (Pritchard et al. 1994; King et al. 2001). Other drugs for which UGT isoforms catalysing their glucuronidation in rats has not yet been characterized could also be metabolized by this enzyme. For example, carbamazepine is glucuronidated by the isoform UGT2B7 in humans, a recognized orthologue of the UGT2B1 in rats. In rat hepatocyte suspensions, this pharmaceutical showed a high inhibitory potency on the biotransformation of both nonylphenol and bisphenol A. Other drugs causing an inhibition of pollutants metabolism such as diclofenac, ibuprofen, naproxen, and valproic acid are also glucuronidated by the UGT2B7 in humans (Kiang et al. 2005). The fourth highest inhibition in the present study was observed with incubations containing mefenamic acid, a drug that has been found to inhibit the glucuronidation of 3′-azido-3′-deoxythymidine (AZT) by UGT2B7 in human microsomes with a \(K\) of 0.3 \(\mu\)M (Mano et al. 2007). The anti-epileptic drug valproic acid also inhibits competitively the glucuronidation of AZT with a much higher \(K\) of 1.6 mM (Ethell et al. 2003). Therefore, the inhibition of bisphenol A and nonylphenol metabolism in rat hepatocytes by drugs known to be glucuronidated by UGT2B1 (in rats) or UGT2B7 (in humans) is most likely to be, solely or partially, explainable by an enzymatic interaction. Although we have observed similar interactions with naproxen in human microsomes (measured \(K\) of 293 \(\mu\)M; Haddad et al. 2009), the relevance of all these observations from this study in rats to the human situation remains to be elucidated since nonylphenol and bisphenol A have been shown to be metabolized mainly by the UGT2B15 isoform, and to a lesser extent UGT2B7, in humans (Hanioka et al. 2008). The use of ethanol as carrier solvent (1% of incubation medium) may have slightly contributed to observed differences but should have a marginal effect based on data by Uchaipichat et al. (2004) where 1% ethanol caused less than 10% reductions on UGT2B7 and UGT2B15 activities.

The strong inhibitions observed with naproxen, salicylic acid, and carbamazepine in rat hepatocyte suspensions prompted their use in microsomal...
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incubations in order to characterize the mechanisms underlying these inhibitions. The glucuronidation of nonylphenol and bisphenol A in the presence of these drugs was assessed with the use of rat liver microsomes. The biotransformation of nonylphenol and bisphenol A by microsomes was inhibited by naproxen and carbamazepine. For both these drugs, the assumption of competitive inhibition yielded the
highest non-linear regression $R^2$ values when using the Enzyme Kinetic module of the Sigmaplot software. In co-incubations with naproxen, the inhibition constant $K_{\text{app}}$ for nonylphenol (581.7 µM) and bisphenol A (848.3 µM) were close to the maximum naproxen concentrations (305-473 µM) found in human blood after administration of a 1000 mg controlled-release tablet in postprandial volunteers (Mroszczak et al. 1988). This finding could be critical in risk assessment for these endocrine disruptors. Further investigations are required to assess adequately if chronic consumption of naproxen could significantly decrease the metabolism of these pollutants in liver and intestinal tissues (Inoue et al. 2003) and consequently result in higher internal exposures. The $K_{\text{app}}$ values found in co-incubations with carbamazepine were over an order of magnitude above the maximum concentration in human blood documented in the literature. A significant metabolic interaction is therefore unlikely to happen at therapeutic levels.

Although salicylic acid displayed a high inhibition of bisphenol A and nonylphenol metabolism in hepatocytes, no inhibition was observed liver microsomes. Salicylic acid is primarily metabolized to salicylic acid in man and rat. The generation of salicyl acyl glucuronide and salicyl phenolic glucuronide is only a secondary pathway that increases in importance as the doses get higher (Patel et al. 1990). Therefore, it is hypothesized that mechanisms other than direct enzymatic competitive inhibition might be involved. Among these possible mechanisms, salicylic acid lowering of pH from 7.5 to 7.2 in hepatocyte suspensions might partially explain the inhibition but is very unlikely to be the only factor. Further experiments are needed in order to address the possible disruption of UDPGA homeostasis or transport by either salicylic acid or one of its metabolites. In addition, the impact of salicyluric acid, the primary salicylic acid metabolite, on glucuronidation rates of bisphenol A and nonylphenol should be assessed. Because nonylphenol and bisphenol A are lipophilic (that is, logP of 3.50 and 5.76, respectively) and non-ionic at physiological pH (that is, $pK_a$ approximately 10 for both), it is unlikely that the high level of inhibition (approximately 90%) observed in hepatocytes in the presence of salicylic acid would be due to inhibition of cellular uptake, since such compounds easily diffuse across the cell membrane.

The measured $K_{\text{app}}$ for nonylphenol (193.2 µM) is in accordance with a previously published value of 250 µM (Daidoji et al. 2003). On the other hand, published measured $K_m$ values for bisphenol A are three- to fourfold lower than the present paper’s (114.4 µM; 25 µM (Yokota et al. 1999), 27 µM (Elsby et al. 2001), and 44 µM (Kuester & Sipes 2007). Discrepancy among results may stem from differences in rat strain and/or gender; Wistar female rats were used by Elsby et al. (2009), Yokota et al. (1999) used Wistar male rats, whereas the present authors used male Sprague-Dawley rats. Another possible explanation for the divergence with results from Kuester & Sipes (2007), who used Sprague-Dawley male rats as we did, is the fact that their measurement was made with hepatocytes suspensions and not liver microsomes. Differences in non-specific binding due to difference in microsomal concentrations may also contribute to the observed differences.

In conclusion, it is suggested that the inhibition of bisphenol A and nonylphenol glucuronidation by drugs may occur at elevated therapeutic plasma levels based on the data obtained in the present in vitro studies. Further studies in with human material are warranted to confirm and better characterize the interaction potential of drugs on bisphenol A and nonylphenol. Risk assessment should therefore include the use of drugs as a potential factor increasing the internal levels of pollutants. Particularly, chronic use of drugs during critical time windows of development, periods of life at which endocrine disrupters are susceptible of triggering enhanced toxic effects, should be carefully addressed. This study showed the importance of further investigating drug-pollutant interactions. Additional studies are planned in order to screen for other metabolic interactions between drugs and environmental pollutants, and to characterize identified inhibition with human tissues.

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Declaration of interest

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References


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