Analysis of the Lidocaine Metabolite 2,6-Dimethylaniline in Bovine and Human Milk

Neil W. Puente and P. David Josephy*

Guelph-Waterloo Centre for Graduate Work in Chemistry (GWC2), Department of Chemistry and Biochemistry, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Abstract

2,6-Dimethylaniline (2,6-xylidine; 2,6-DMA) is a nasal carcinogen in rats. Humans may be exposed to this compound via several routes: 2,6-DMA is found in cigarette smoke; it is a pharmacologically inactive metabolite of some drugs (e.g., the local anesthetic lidocaine) and pesticides (e.g., metalaxyl); and it is an impurity in technical grade metalaxyl. The potential transfer of 2,6-DMA from mother to nursing infant via milk is of toxicological concern. Solid-phase microextraction with separation and detection using gas chromatography–mass spectrometry was optimized and used for the analysis of 2,6-DMA in milk. 2,6-DMA-δ9 was synthesized and used for quantitation by the isotope ratio method. At a concentration of 5 ppb 2,6-DMA, the method detection limit was 0.20 ppb, and the relative standard deviation was 3.6%. Samples of milk were obtained from bovines administered lidocaine (2.9–3.9 mg/kg) during surgery. A breast milk sample was also obtained from a human donor who received 36 mg lidocaine during dental work. 2,6-DMA was present at levels ranging from 14.5 to 66.0 ppb in bovine milk and was detected at 1.6 ppb in the human milk sample. Our results demonstrate that 2,6-DMA, formed by the metabolism of lidocaine, is transferable to bovine and human milk.

2,6-Dimethylaniline (2,6-DMA; chemical structures are shown in Figure 1) has been identified as a nasal carcinogen in rat long-term feeding studies (1). A recent study (2) indicates that 2,6-DMA may also act as a tumor promoter, enhancing the effect of initiating carcinogens such as nitrosamines. Although there is no direct evidence that 2,6-DMA is a human carcinogen, exposure to this aromatic amine, which may occur via multiple routes, is of concern.

2,6-DMA is a metabolite of lidocaine. Lidocaine (2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide) is commonly used as a local anesthetic in medicine and dentistry; is indicated for the treatment of cardiac arrhythmia (3); and, administered intranasally, is effective for the treatment of migraine headaches (4–6). 2,6-DMA metabolite excreted in the urine represents about 1% of the administered dose of lidocaine in humans (7); 4-hydroxy-2,6-dimethylaniline, the major urinary metabolite of lidocaine, is likely formed by further oxidation of 2,6-DMA (8). Other potential routes of exposure to 2,6-DMA include metabolism of the fungicide metalaxyl (9); metabolism of the veterinary tranquilizer xylazine, which may remain as a residue in meat (10); and exposure to cigarette smoke, including side-stream smoke (11).

The transfer to human milk of lidocaine and its de-ethylated metabolite monoethyl-glycinexylidide (MEGX) has been demonstrated (12–14). Our laboratory has previously developed methods for the analysis of aromatic amines in biological fluids (15,16), based on the technology of solid-phase microextraction (SPME) (17), with separation and detection using gas chromatography–mass spectrometry (GC–MS). SPME has also been used for the analysis of lidocaine in biological matrices (18). We have now developed an SPME-GC–MS method for analysis of 2,6-DMA in milk. The results presented here demonstrate that 2,6-DMA produced from the metabolism of lidocaine is transferred to bovine and human milk.

Figure 1. Structures of 2,6-DMA, lidocaine, and related compounds.
Experimental

Synthesis of 2,6-DMA-d$_9$ standard

2,6-DMA-d$_9$ was synthesized by nitration and reduction of m-xylene-d$_{10}$ (CDN Isotopes, Inc., Pointe-Claire, QC, Canada) and subsequent purification of the desired product. m-Xylene-d$_{10}$ (1.0 mL), water (0.3 mL), sulfuric acid (1.5 mL), and nitric acid (0.90 mL) were mixed in a round bottom flask. The solution was refluxed at 59°C for 6 min. The product (nitro-xylene isomers) was reduced with granulated tin (2.30 g) and hydrochloric acid (4.76 mL) and vigorously stirred for 20 min at 58°C. The product mixture was dissolved in methanol prior to high-performance liquid chromatography (HPLC) purification.

Purification of the 2,6-DMA-d$_9$ standard was carried out on a Hewlett-Packard (now Agilent Technologies, Wilmington, DE) HP 1050 HPLC with Supelcosil PLC-SI column (25 cm x 4.6-mm i.d., 15-µm particle size, Supelco, Sigma-Aldrich Canada), using conditions adapted from those of Schmeltz et al. (19). The peak corresponding to 2,6-DMA-d$_9$ (identity confirmed by GC–MS, see GC–MS section) was collected, quantitated by UV absorbance, and used to prepare the internal standard dilution.

Milk samples

Bovine milk was obtained from animals treated at the Ontario Veterinary College (Guelph, ON, Canada). Samples were kept frozen at -70°C until analyzed. Samples were thawed and then sonicated for 5 min at 25°C. An aliquot of milk (8 mL) was pipetted into a Corning 15-mL conical centrifuge tube, spiked with the 2,6-DMA-d$_9$ internal standard, and mixed thoroughly. Samples were centrifuged (4000 rpm, 10 min, 4°C), causing the fat to separate. The fat plug was removed by use of a cotton swab. An aliquot of the skim milk (5 mL) was then pipetted into a 7-mL Supelco clear vial with hole cap and PTFE/silicone septum, prepared as described here, for SPME sampling.

SPME

The SPME sampling protocol was adapted from our earlier method (15). The SPME fibers (CW/DVB coating) and the SPME holder assembly were purchased from Supelco. One milliliter of 12M KOH solution was pipetted into a 7-mL vial containing 1.403 g NaCl (Fisher Scientific, Nepean, ON, Canada), an octagonal stirbar (1/2 in. x 1/8 in.), and 5 mL skim milk. Vials were stirred (1500 rpm) at 60°C using a digital hotplate/stirrer (Dataplate® PMC 730 series, Fisher Scientific). Vials were equilibrated (3 min) before the SPME fiber was inserted into the headspace. Headspace extraction was carried out for 30 min; the fiber was then retracted and injected into the GC. Thermal desorption time was 1 min.

GC–MS

Analyses were conducted using a Hewlett-Packard GC (5890 series) coupled to an HP 5971 mass selective detector in the selected ion monitoring mode. The carrier gas was He at 20 cm/s. A silanized 0.75-mm insert was used. The column (1%(5%-diphenyl-(95%)-dimethylsiloxane copolymer, 30 m x 0.25-mm i.d., 0.25-µm film thickness, DB-5MS, J&W Scientific, Folsom, CA) was held at 60°C for 1 min and then ramped to 140°C (at 10°C/min, no hold time at 140°C) and finally to 250°C (25°C/min, 1-min hold). Total run time was 14.40 min. The injector temperature was set to 210°C, and the detector was set to 300°C. The dwell time for SIM mode was 10 ms. Ion acceptance range was -0.3 to +0.7 of nominal mass. Daily midmass autotunes with perfluorotributylamine were carried out. Liquid injections (1 μL) were made in the splitless mode. The head pressure was 10 psi. For SPME injections, the purge was started at 1 min.

Figure 2 shows the mass spectrum of 2,6-DMA-d$_9$. The parent ion is m/z 112, and loss of a CD$_3$ gives daughter ion m/z 112. For 2,6-DMA, the corresponding ions occur at m/z 121 and 106, respectively.

Calibration curves, precision, and method detection limit

Calibration curves for 2,6-DMA in bovine milk were generated over the range 0.5-20 ppb. To test day-to-day variation, three separate calibration curves were generated on different days. The precision and method detection limit (MDL) were determined from 10 replicate extractions of 5 ppb 2,6-DMA + 5 ppb 2,6-DMA-d$_9$. For quantitation of 2,6-DMA in milk, samples were spiked with the internal standard (2,6-DMA-d$_9$) to a final concentration of 5 ppb.

Milk analysis

- **Bovine milk**: Milk samples (approximately 30 mL) were taken from each of seven Holstein cows. Samples were obtained before surgery and at a single time 2.5-6.0 h after lidocaine injection.
- **Human milk**: Control milk samples from 15 human donors not exposed to lidocaine (or other known sources of 2,6-DMA) were obtained with informed consent. A single human milk sample was obtained from a woman who had received 36 mg lidocaine by local injection during dental work; the sample was collected 6 h after the lidocaine injection.

Results and Discussion

**SPME analysis**

Based on preliminary optimization studies, we selected the SPME conditions (fiber type, temperature, and time) described

![Figure 2. Mass spectrum of 2,6-DMA-d$_9$.](image-url)
in the Experimental section, as providing optimum extraction of 2,6-DMA in a reasonable period of time. A typical calibration curve (0.5–20 ppb) is shown in Figure 3. Calibration curves were linear ($r^2 > 0.99$). The day-to-day variation was estimated as 11.0%, based on calibration curves generated on three days. Relative standard deviation (10 replicate extractions, 5 ppb) was 3.6%. For a 99% confidence interval, the Student’s t value is 2.821 (n = 10 with 9 degrees of freedom). The MDL, calculated by multiplying this value by the standard deviation for the 10 replicates (0.0725), was 0.20 ppb.

2,6-DMA in bovine milk

Milk samples obtained before lidocaine injection were uniformly negative for 2,6-DMA and all samples obtained post-injection were positive, with 2,6-DMA concentrations ranging from 14.5-66.0 ppb. Typical blank (pre-injection) and positive (post-injection) GC chromatograms are shown in Figure 4. The concentrations of 2,6-DMA were calculated from the calibration curve. Table I reports, for each sample, the lidocaine dose administered, the time of milk sampling, the measured concentration of 2,6-DMA, and the protein and lipid content. Levels of 2,6-DMA in milk varied over an approximately fourfold range. There was no obvious relationship between milk 2,6-DMA level and dose, time of sampling, or milk protein or fat levels, although the number of samples examined was limited.

2,6-DMA in human milk

Fifteen milk samples from donors with no known exposure to lidocaine or 2,6-DMA were analyzed and none contained detectable levels of 2,6-DMA. The single milk sample obtained from a donor who had received an injection of 36 mg of lidocaine contained 1.6 ppb 2,6-DMA. A typical blank chromatogram and the chromatogram for the positive sample are shown in Figure 5. (Although it is not possible to state with certainty that the 2,6-DMA is lidocaine-derived, this seems very likely, because the compound was never found in unexposed control samples.)

Lidocaine and certain metabolites, such as MEGX, have previously been detected in blood, urine, and human milk. In the present work, we have shown that headspace SPME, using the CW/DVB fiber, coupled with GC–MS provides a rapid, reliable, and sensitive method for the analysis of the lidocaine metabolite 2,6-DMA in animal and human milk. In recent publications, Abdel-Rehim and colleagues (20,21) have also applied SPME, in combination with GC or HPLC–tandem electrospray mass spectrometry, to the analysis of lidocaine and DMA in spiked samples of human plasma and urine.

<table>
<thead>
<tr>
<th>Case</th>
<th>Dose (mg/kg)</th>
<th>Post-injection sampling time (h)</th>
<th>2,6-DMA (ppb)</th>
<th>Protein (g/L)</th>
<th>Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>261</td>
<td>3.9</td>
<td>3</td>
<td>14.5</td>
<td>40.5</td>
<td>10</td>
</tr>
<tr>
<td>259</td>
<td>3.5</td>
<td>4</td>
<td>66.0</td>
<td>50.4</td>
<td>11</td>
</tr>
<tr>
<td>254</td>
<td>3.4</td>
<td>2.5</td>
<td>47.9</td>
<td>28.9</td>
<td>13</td>
</tr>
<tr>
<td>260</td>
<td>3.2</td>
<td>6.5</td>
<td>16.8</td>
<td>46.4</td>
<td>6</td>
</tr>
<tr>
<td>255</td>
<td>3.0</td>
<td>3.5</td>
<td>36.8</td>
<td>36.6</td>
<td>4</td>
</tr>
<tr>
<td>258</td>
<td>3.0</td>
<td>2.5</td>
<td>24.0</td>
<td>33.3</td>
<td>7</td>
</tr>
<tr>
<td>256</td>
<td>2.9</td>
<td>9</td>
<td>39.7</td>
<td>31.0</td>
<td>8</td>
</tr>
</tbody>
</table>

* Milk protein and fat concentrations were measured using the modified Lowry assay (22) and modified Folch extraction (23), respectively.
The presence of 2,6-DMA as a lidocaine metabolite in milk demonstrates that this xenobiotic can reach the mammary tissue. The observation of 2,6-DMA in the milk of a nursing mother treated with lidocaine suggests that the drug metabolite can reach the milk and be ingested by nursing infants. For a compound to pass from the maternal circulation to the milk, it must cross multiple membrane barriers. Compounds that are relatively lipophilic, low molecular weight, un-ionized, and not strongly protein-bound will diffuse into the breast milk most readily. 2,6-DMA satisfies all of these criteria.

Conclusions

Treatment of nursing mothers with lidocaine or other compounds which may give rise to 2,6-DMA is likely to be a potential route for exposure of infants. Although the levels of 2,6-DMA exposure are far below those which are associated with its carcinogenicity in experimental animals, any exposure of infants to the compound should probably be avoided, if possible. Monitoring human exposure requires the development of reliable methods for analysis of 2,6-DMA in complex matrices.

Acknowledgments

We are grateful to Dr. Donald Trout, Ontario Veterinary College, who conducted the bovine anesthesia and collected the bovine milk samples. Dr. Perry Martos, Lab. Services, and Dr. Julie De Merchant, Chemistry and Biochemistry, University of Guelph, generously provided advice and access to analytical instruments. We also thank Dr. Martos for critical reading of the manuscript. Finally, we wish to thank Patricia Solbeck for measuring the milk protein and lipid levels. Funding was provided by the Toxic Substances Research Initiative (Health Canada) and NSERC Canada.

References


Figure 5. Analysis of 2,6-DMA in human milk. A typical blank GC chromatogram (A) and the GC chromatogram for the positive sample obtained from a donor who had received an injection of 36 mg lidocaine (B) are shown. The vertical axis represents relative detector response, and the blank chromatograms are shown on an expanded scale.

Headspace SPME coupled with GC-MS is a rapid and sensitive method for analysis of 2,6-DMA in milk.


Manuscript received March 2, 2001; revision received June 11, 2001.