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Urinary Excretion of Cyclohexanediol, a Metabolite of the Solvent Cyclohexanone, by Infants in a Special Care Unit

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Using gas chromatography–mass spectrometry, we investigated the urinary excretion of organic acids of 278 newborn babies in a special care unit to obtain reference data and monitor metabolism. In 101 of 584 urine samples analyzed, we found isomers of cyclohexanediol. trans-1,2-Cyclohexanediol was always most abundant, with small amounts of 1,3- and 1,4-cyclohexanediol and, sometimes, traces of cis-1,2-cyclohexanediol. Glucuronide conjugates were not detected. The probable source was the solvent cyclohexanone, which was found as a contaminant of intravenous dextrose and the parenteral feeding solution, and was also leached into the infusion fluids from the administration set. We recovered 0.89 mg (range 0.74–0.98 mg, n = 5) of cyclohexanone from 150 mL of dextrose pumped through the infusion apparatus over 24 h, the normal rate for a 1-kg premature baby. Although this is well below toxic doses reported for mature animals, more data are needed for the newborn, particularly preterm infants who have a decreased capacity for glucuronide conjugation.

Additional Keyphrases: newborns · toxicology · chromatography · gas · mass spectroscopy · parenteral nutrition · organic acids

We have been investigating the urinary excretion of organic acids by neonates since 1985. In our first study, an incidental finding was that premature babies who were fed by the intravenous route excreted a family of unusual compounds, identified as isomers of cyclohexanediol (1), which are not known to be produced during normal intermediary metabolism. Cyclohexanediol was not detected during milk feeding and was not present in any of the intravenous fluids or vitamin and mineral supplements used, including samples obtained from an infusion system. It was not produced as an artifact in the analytical procedure, and its presence could not be linked to antibiotic therapy. We inferred that the compound was produced by endogenous modification of some component of the intravenous regimen. In a second study, begun in 1988, we again found cyclohexanediol in urine from babies receiving intravenous dextrose or parenteral nutrition. This led us to review the data from both studies. A likely source of the diol was cyclohexanone, which is used as a solvent for polyvinyl chloride (PVC) in many medical devices.1 Two groups have reported this compound in fluids from intravenous infusion bags (2, 3). Recently, three adult men industrially exposed to cyclohexanone by inhalation were found to metabolize the compound to cyclohexanol and to isomers of cyclohexanediol, which were excreted in urine (4). We therefore investigated the infusion system and fluids used in the special care unit for possible contamination with cyclohexanone.

Materials and Methods

Patients

We studied, with parental consent, two groups of newborn babies admitted to the special care baby unit of the Princess Anne Hospital, Southampton, U.K. The first group comprised 86 babies admitted from June to November 1985; 23 were of 25–32 weeks of gestation, 29 of 33–36 weeks, and 34 of 37 weeks or more of gestation. The second group comprised 192 babies admitted from July 1988 to December 1989: 55 of 26–32 weeks, 60 of 33–36 weeks, and 77 of 37 weeks or more of gestation.

In the first study, random (untimed) urine samples were collected into a bag, once during the first 48 h postpartum and then at weekly intervals until discharge. In the later study, random urine samples were collected once or twice during the first 48 h postpartum, and once at 7–10 days postpartum. Samples were frozen immediately, without preservative, and stored at –20 °C until analysis. The study was approved by the Local District Ethical Committee.

The babies received normal clinical care. Those who were too sick for oral feeding received intravenous fluids. During the first three days, this comprised dextrose and electrolytes. As the clinical condition improved, oral milk was introduced and the volume of intravenous fluid was reduced progressively during the first week. Babies who required assisted ventilation were started on parenteral nutrition, generally on the fourth day postpartum. These were all born before 33 weeks of gestation except for one baby delivered at 34 weeks of gestation. The full regimen, established gradually over five days, comprised Vamin 9 glucose, 10% Intralipid (fat suspension), Ped-el minerals, Vitlipid fat-soluble vitamins, and Solivito water-soluble vitamins (all from Kabivitrum Ltd., Middlesex, U.K.) and 100 g/L dextrose solution, infused at 150 mL/kg of body weight daily. Vamin 9 glucose was omitted if renal or liver damage was present, and Intralipid if there was liver damage or if plasma bilirubin exceeded 150 μmol/L. Dextrose solution was infused from PVC bags to which electrolytes had been added. The aqueous component of the parenteral nutrition regimen was prepared in the hospital pharmacy: dextrose (100 g/L), Vamin 9 glucose, minerals, electrolytes, water, and Solivito were dispensed into ethyl vinyl acetate bags. All intravenous fluids were administered through a disposable plastic burette, bacteriological filter, and intravenous cannula in sequence. Intralipid and Vitlipid were added to the infusion as it emerged from the filter. Except for the cannula, the complete infusion system was replaced daily.

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Analytical Reagents and Methods

Cyclohexanol, 2-hexanone, trans-1,2-cyclohexanediol, and mixtures of the cis and trans isomers of 1,2-cyclohexanediol, 1,3-cyclohexanediol, and 1,4-cyclohexanediol were from Aldrich Chemical Co. (Gillingham, U.K.). Cyclohexanone and solvents (all Analar grade) were from BDH Ltd. (Poole, U.K.). β-Glucuronidase from Helix pomatia (130 kU of β-glucuronidase (EC 3.2.1.31) and 3100 U of sulfatase (EC 3.1.6.1) per millilitre) and all other chemicals were from Sigma Chemical Co. (Poole, U.K.). Water was glass-distilled and then de-ionized. Urinary creatinine was measured by an alkaline picrate method (5).

Analysis of urinary organic acids. In the first study, we extracted 1-mL urine samples with hydroxylamine hydrochloride, acidified them to pH 1.0 with 5 mol/L hydrochloric acid, saturated with sodium chloride, then successively extracted with ethyl acetate and diethyl ether. The extracts were derivatized with 200 μL of bis(trimethylsilyl)trifluoroacetamide (BSTFA). Acids were separated on a 25 m × 0.33 mm (i.d.) BP1 (OV-101 equivalent) fused silica capillary gas-chromatography column (Scientific Glass Engineering, Milton Keynes, U.K.), by using a temperature program of 50 °C for 5 min, then increasing by 6 °C/min to 260 °C. Typically, 0.2–0.8 μL was injected, with use of a cooled on-column technique. Acids were quantified from their integrated peak areas with reference to tetraocosane internal standard. Unknown compounds were identified by combined gas chromatography/electron impact mass spectrometry (GC-MS) (6).

In the second study, we extracted the volume of urine equivalent to containing 1 μmol of creatinine. After dissolving the extracted acids in 200 μL of BSTFA, we injected 1 μL of the derivatives (equivalent to 5 nmol of creatinine), at a 20:1 split ratio, onto the GC column.

Analysis of cyclohexane. The majority of premature babies receiving intravenous fluids weighed about 1 kg and received 150 mL of fluid per kilogram of body weight daily. We therefore analyzed 150 mL of fluid as representative of the volume passing through an infusion system in 24 h. Fluid was acidified to pH 1.0 with hydrochloric acid and extracted four times with 25 mL of diethyl ether, and the combined extracts were carefully evaporated to 1 mL under nitrogen at room temperature. Because of the volatility of cyclohexanone (normal boiling point 155 °C), TMS derivatization was omitted and 1 μL of the concentrated extract was injected (split injection) onto the GC column, under the conditions described above. Analytical recovery of 250 μg of standard cyclohexanone added to 150 mL of water was 23 ± 2% (n = 4). For quantitative studies, we added 250 μg of 2-hexanone as internal standard to the infusion fluids before extraction, and quantified cyclohexanone by comparison of their integrated peak areas. Analytical recovery of 250 μg of 2-hexanone from water (24 ± 3%, n = 4) was similar to that of cyclohexanone. The relative response factor of cyclohexanone relative to 2-hexanone was 1.03. No cyclohexanone was detectable in procedural blanks prepared with distilled de-ionized water.

Analysis for cyclohexanol in urine. Three or 4 mL of urine, depending upon availability, was acidified to pH 1.0 and extracted four times with 2.5 mL of diethyl ether. The combined ether extracts were evaporated to 1 mL under nitrogen at room temperature, and 3 μL of the extract was injected onto the GC column under the conditions described previously. As little as 2 ng (mean + 2 SD of baseline noise) of cyclohexanol was detectable by GC (40 ng injected, 20:1 split ratio).

Analysis of urine for glucuronide conjugates of cyclohexanediol and cyclohexanol. We used three methods:

(a) urine, acidified to pH 1.0, and extracted with ethyl acetate, was analyzed by thin-layer chromatography on silica gel with benzene/acetate acid/water (70/29/1 by vol) for development, and naphthoresorcinol as stain agent (7).

(b) 1 mL of urine was mixed with an equal volume of 5 mol/L hydrochloric acid and heated for 1 h at 100 °C. Split samples of hydrolyzed and nonhydrolyzed urine were extracted with ethyl acetate and diethyl ether, derivatized with BSTFA, and analyzed for cyclohexanediol by GC as before; and

(c) urine acidified to pH 5.0 was incubated with β-glucuronidase (approximately 3500 U/mL of urine) at 37 °C overnight. Split samples of hydrolyzed and nonhydrolyzed urine were extracted and analyzed for cyclohexanediol and cyclohexanol.

Results

We first identified cyclohexanediol in 1985 in nine urine samples collected from babies who were receiving parenteral nutrition (2). In the organic acid profiles obtained by capillary GC, there was a large unknown peak with the same retention time as phosphate, methylene unit (MU) 12.77 (peak 3 in Figure 1). The electron impact mass spectrum of the compound is shown in Figure 2A. After a literature search, we tentatively identified it as the di-TMS derivative of 1,2-cyclohexanediol (molecular mass 260 Da).

Only trace amounts of phosphate were noted. The mass spectra of two other unknown compounds giving rise to small peaks at MU 12.68 and 13.26 (peaks 2 and 5 in Figure 1) are shown in Figures 2B and C, respectively. These were thought to be the di-TMS derivatives of the isomers 1,3- and 1,4-cyclohexanediol. Comparison of the mass spectra and retention times with authentic standards confirmed the identity of all these compounds. Peak 3 was assigned definitely to the di-TMS derivative of the trans isomer of 1,2-cyclohexanediol, for which a commercial standard was available. The di-TMS derivative of cis-1,2-cyclohexanediol eluted earlier, at MU 12.67. A commercial mixture of cis- and trans-1,3-cyclohexanediols, after derivatization, had peaks at MU 12.67 and 13.13, respectively, from which we would conclude that peak 3 is 1,2-cyclohexanediol.
deduced that the compound identified in urine samples (peak 2) was likely to be the cis isomer. A mixture of cis- and trans-1,4-cyclohexanediols (di-TMS derivatives) had peaks at MU 13.19 and 13.27, respectively, and it seems likely that the trans isomer was the form (peak 5) present in urine. Mean analytical recovery of trans-1,2-cyclohexanediol (20 μg/mL added to urine) was 99.8% (CV 1.96%, n = 5), and 250 pg was detectable by GC (5 ng injected, 20:1 split ratio) in the standard procedure.

We have reviewed all the data from a total of 584 urine samples from 278 babies from the 1985 and 1986 studies. Cyclohexanediol was identified in 101 samples, all of which were collected from babies receiving an intravenous infusion (Table 1). trans-1,2-Cyclohexanediol was always the most abundant isomer, but this could not be quantified accurately in most samples because of small amounts of co-eluting phosphate. The highest concentration was 30 mmol/mol of creatinine in the sample shown in Figure 1, in which no phosphate was detectable. This isomer was always accompanied by lower concentrations of 1,3- and 1,4-cyclohexanediol detectable by GC-MS, but often in amounts below the level for reliable quantification. By GC-MS, cis-1,2-cyclohexanediol was detectable in some samples with high concentrations of the trans-1,2 isomer (see Figure 1). 1,4-Cyclohexanediol (MU 13.27) did not co-elute with any other compound in neonatal urine, and detection of even a small peak in the GC profiles was a reliable indication of the presence of the other isomers.

Table 1 shows that the diols were found in samples from babies who were receiving either intravenous dextrose or parenteral nutrition. More positive samples were found with the latter, however, perhaps because intravenous infusion had been in progress for longer. Diols were detected in 80 of 238 samples from babies of <33 weeks of gestation compared with 21 of 346 samples from the other babies. The immature infants required intravenous therapy more often, and for longer.

The cyclohexanediol we detected was unconjugated. To determine whether the babies also excreted glucuronide conjugates of the diol, we subjected to acid hydrolysis two urine samples containing significant amounts of diol. A third sample, and a pool of urine samples of high diol content, were hydrolyzed with β-glucuronidase. There was no more free cyclohexanediol in the hydrolyzed samples than in the nonhydrolyzed samples. No glucuronides were detected by thin-layer chromatography of three urine samples containing high concentrations of free diol.

Five individual urine samples and five pools of urine that contained cyclohexanediol were analyzed for cyclohexanol with and without hydrolysis with β-glucuronidase. (The generally small volume of urine samples collected necessitated pooling.) Free cyclohexanol was not detected in any sample. A small peak of the compound was identified tentatively by GC after hydrolysis of one of the pooled samples, but could not be confirmed by GC-MS, probably because of its very low concentration.

The infusion fluids and infusion system were investigated as possible sources of cyclohexanone. In this preliminary experiment, accurate quantification was not attempted, and each observation was made only once. We found that both 100 g/L dextrose and the intravenous feeding mixture (which includes a large component of 100 g/L dextrose) contained cyclohexanone before being introduced into the administration set. In addition, substantial amounts of cyclohexanone were leached from the infusion system when both water and the parenteral feeding solution were pumped through at 150 mL/24 h. The burette, rather than the filter, was the main source of the compound.

### Table 1. Babies Studied and Urine Samples Analyzed for Cyclohexanediol

<table>
<thead>
<tr>
<th>Type of Infusion</th>
<th>Samples with cyclohexanediol</th>
<th>Infusion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion</td>
<td>No.</td>
<td>Median (range) duration of infusion, days</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>(range) duration of infusion, days</td>
</tr>
<tr>
<td>1985</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-32</td>
<td>23</td>
<td>12 (2-46)</td>
</tr>
<tr>
<td>33-36</td>
<td>29</td>
<td>7 (1-9)</td>
</tr>
<tr>
<td>37+</td>
<td>34</td>
<td>9 (7-10)</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>57</td>
</tr>
<tr>
<td>1986-89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-32</td>
<td>55</td>
<td>7 (1-10)</td>
</tr>
<tr>
<td>33-36</td>
<td>60</td>
<td>7 (3-8)</td>
</tr>
<tr>
<td>37+</td>
<td>77</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>192</td>
<td>44</td>
</tr>
</tbody>
</table>

* Intravenous fluid infused at the time of collection: Dex = dextrose; PN = parenteral nutrition.
We then undertook an experiment to quantify the amount of cyclohexanone which a 1-kg preterm baby would receive during an infusion of 150 mL of 100 g/L dextrose over 24 h. We set up five complete infusion systems, pumping through each 100 g/L dextrose, from the normal PVC bag supplied, and collecting the fluid in a volumetric flask. The amount of cyclohexanone in the 24-h infusates ranged from 0.74 to 0.98 mg; mean 0.89 mg (7.5–10.0 μmol, mean 9.1 μmol).

Discussion

The cyclohexanediol isomers identified are unusual compounds that are not known intermediates of endogenous metabolic processes. They have not, to our knowledge, been reported before in urine from sick infants or children. We deduced that they were formed endogenously from some constituent or contaminant of fluids administered intravenously. The solvent cyclohexanone (2, 3) was a likely candidate.

The metabolic pathway by which cyclohexanone could be converted to cyclohexanediol is shown in Figure 3. Observations in animals support this sequence. Rabbits (8) and dogs (9) fed cyclohexanone orally (248 and 284 mg/kg, respectively) reduced it to cyclohexanol, which was excreted as the glucuronide conjugate. This was apparently the main metabolite of cyclohexanone. When rabbits were fed cyclohexanol (260–270 mg/kg), 65% was excreted as cyclohexanol–glucuronide conjugate, and 6% as the glucuronide conjugate of trans-1,2-cyclohexanediol (8). Thus an enzyme pathway exists in vivo by which cyclohexanone could be converted through cyclohexanol to trans-1,2-cyclohexanediol, the most abundant isomer that we found. Two reports of exposure to the solvent indicate that this reaction sequence also occurs in humans. One adult who drank cyclohexanone excreted cyclohexanol (10), and three men who inhaled cyclohexanone excreted cyclohexanol, trans-1,2-cyclohexanediol, and trans-1,4-cyclohexanediol (4). Cyclohexanone is reduced to cyclohexanol at pH 7.0 by human liver alcohol dehydrogenase (EC 1.1.1.1) (11), an enzyme demonstrated to be present in human fetal liver from as early as two months of gestation (12). Hydroxylation of foreign compounds by the microsomal mixed-function oxidase system is a common detoxification process. Microsomes exhibit high hydroxylating activity for cyclohexane (13), which is structurally similar to cyclohexanone and is metabolized to cyclohexanol and trans-1,2-cyclohexanediol in rabbits (8). An intact and active microsomal oxidizing system has been demonstrated in the human fetus early in gestation (14, 15).

In contrast to reports that cyclohexanol was the major metabolite of cyclohexanone (8–10), we were able to (tentatively) identify a small amount of cyclohexanol in only one pooled sample with a high concentration of cyclohexanediol. One possible explanation was the insensitivity of our analytical method. Recovery from urine with diethyl ether was probably poor, and losses were likely during extraction and chromatography. Nevertheless, we were able to detect as little as 2 ng of cyclohexanol by GC and, if cyclohexanol were the major metabolite, we should have found it. However, Flek and Sedivec (4) reported recently that urine collected from three adults after exposure for 8 h to cyclohexanone (415 mg/m²) contained trans-1,2-cyclohexanediol as the main metabolite (68.4%), followed by trans-1,4-cyclohexanediol (25.1%), and only 3.5% of cyclohexanol. They found cis isomers of cyclohexanediol in only minute amounts. Cyclohexanol disappeared from the urine after 24 h, whereas excretion of cyclohexanediol continued even after 10 days (4). This metabolite profile more closely resembles that in neonates. Differences in excreted products may relate to the dose of cyclohexanone received.

In all the reported studies, cyclohexanol and cyclohexanediol were excreted mainly as glucuronide conjugates. We found only free, unconjugated cyclohexanediol in neonatal urine. This is readily explained by decreased activity of microsomal glucuronosyltransferase (EC 2.4.1.17) in neonatal liver, particularly in premature babies (15).

Our study adds to a number of reports of plasticizers or their metabolites—mostly phthalates—found in blood products, urine, and parenteral solutions (3). One study of neonates found an oxidation product of 2-(hydroxyethylthio)benzothiazole in urine, which was a contaminant from intravenous infusion equipment (16). In our study, we found that cyclohexanol was present in 100 g/L dextrose and in the parenteral feeding regimen (probably from the dextrose solution which it contained), and that it was leached into the infusion fluids from a plastic burette included in the administration set. We selected these items for analysis because they were used in our special care unit. However, it is probable that cyclohexanone contamination also occurs from similar items from other manufacturers.

Cyclohexanone is a useful solvent. The important practical question arising from our findings is whether toxicity is likely in the quantities that babies receive by infusion, estimated as almost 1 mg per day for a 1-kg baby. In both acute and chronic animal studies, cyclohexanone had a moderate to low toxicity by oral, intraperitoneal, and intravenous routes (17). Intravenous injection of 5 mg/kg of body weight daily three days a week for three weeks did not have adverse effects on rabbits or guinea pigs (18). Injection of 284 mg/kg intravenously caused central nervous system disturbances in dogs and deaths from respiratory depression in one monkey and one dog, and 568 mg/kg was lethal to rats (9, 19). There is very little information about toxicity in neonates. Addition of cyclohexanone, 10 g/L, to the feeds of mice during pregnancy and lactation significantly increased the mortality, and decreased the growth rate, of their offspring during the first 21 days of life (20).

Fig. 3. Proposed biochemical pathway by which cyclohexanone is converted to cyclohexanediol

Enzymes: 1, alcohol dehydrogenase (EC 1.1.1.1); 2, microsomal mixed-function oxidase (no EC no. assigned)
This finding might indicate increased vulnerability of neonates to exposure to cyclohexanone. A particular worry in preterm neonates is that foreign compounds that compete with bilirubin and drugs for transport proteins and glucuronosyltransferase in liver cells may increase the risk of kernicterus or of drug toxicity (21). We do not know whether cyclohexanone might compete in this way.

Although the amount of cyclohexanone infused is probably too small to be toxic, there is a need for more studies of the effects of this compound in the neonatal period. Perhaps the use of alternative nontoxic material should be considered for the manufacture of intravenous infusion equipment.

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