Excess Serum Osmolality Gap After Ingestion of Methanol: A Methodology-Associated Phenomenon?

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A patient intentionally ingested an unknown amount of methanol and was admitted to the hospital 6 h later. On admission, the methanol concentration in blood was estimated as -134 mmol/L, based on the calculation of the osmolal gap. Intravenous ethanol administration and hemodialysis were promptly started. During hemodialysis, several blood samples were collected for determination of methanol and ethanol concentrations. Initially, we used gas chromatography with split-mode injection of pre-treated serum samples; however, methanol concentrations turned out to be significantly lower than expected, based on calculated osmolal gap values. Because no explanation for the excess serum osmolal gap was apparent, we reanalyzed samples, using head-space gas chromatography. The methanol concentrations measured were significantly higher and osmolal gap values were no longer excessive.

Indexing Terms: gas chromatography with head-space analysis vs split-mode injection compared/toxicology/metabolic acidosis

Metabolic acidosis associated with a high anion gap and high osmolar gap is considered an important laboratory indicator of methanol poisoning (1). The difference between measured and calculated osmolality (Δosmolality, or osmolar gap) permits a rough estimation of alcohol concentrations (2), so that specific therapy is often initiated before results of quantitative methanol determinations are available.

After quantitative determination of the alcohol concentration, the osmolar contribution of a given ethanol or methanol concentration can be calculated and subtracted from the osmolal gap value. The remainder is generally referred to as residual osmolality or excess osmolar gap (EOG) (3). With residual osmolality, one can determine whether the alcohol concentration in a patient’s serum fully accounts for the observed osmolar gap, or whether some other osmotically active substance is present, as indicated by an otherwise unexplained increase in the gap. A value of +10 mosmol/kg has been suggested arbitrarily as the upper limit for EOG in cases of ethanol intoxication (2). However, in a recent retrospective application of this simple rule, as many as 39% of patients presenting with signs of ethanol intoxication were classified as having an abnormally high EOG (3). In another report, Meatherall and Krahn (4) described three cases of methanol intoxication with an extremely high excess serum osmolality gap. EOG values >40 mosmol/kg were observed and, apparently, calculations based on osmolal gap grossly overestimated the amount of methanol present. The compounds possibly involved in such cases of high EOG remain unknown.

Using data obtained in a recent case of severe methanol ingestion, we present further information on EOG and propose a possible explanation for this phenomenon.

Case Report

A 41-year-old man was admitted to the hospital 6 h after intentional ingestion of an unknown amount of methanol. On admission, his respiration, blood pressure, heart rate, body temperature, and Glasgow coma scale rating were normal. No disturbances of vision were present. Relevant laboratory results are summarized in Table 1. The presence of a metabolic acidosis, associated with an increased anion gap (29 mmol/L) and osmolar gap (134 mosmol/kg), was compatible with methanol ingestion. Results of fundoscopic eye examination were normal.

Intravenous ethanol administration was started. A loading dose of 800 mL of a 50 mL/L ethanol solution in 1 h was followed by further administration of 100 mL/h to achieve a serum ethanol concentration between 20 and 30 mmol/L.4 In addition, because of the high serum methanol (95 mmol/L), hemodialysis (artificial kidney hemophane, 1.3 m²; Bella 613, Mirandola, Italy; blood flow rate: 300 mL/min) was started and continued for 8 h, until serum methanol decreased to 14 mmol/L. After dialysis was stopped, a rebound effect of methanol concentration was observed.

The patient was discharged 4 days later in good clinical condition without sequelae.

Materials and Methods

Serum electrolytes, glucose, and urea nitrogen were measured on an Ektachem 700XRC analyzer (Kodak, Vilvoorde, Belgium). Osmolality was measured by freezing-point depression, with an Advanced Instruments Model 3C2 Advanced Cryometric Osmometer (De

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4Conversion factors for alcohol concentrations, mmol/L to g/L: ethanol, 0.046; methanol, 0.032.
Table 1. Relevant laboratory results on admission.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Patient's result</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine, μmol/L</td>
<td>115</td>
<td>44-106</td>
</tr>
<tr>
<td>Na⁺, mmol/L</td>
<td>143</td>
<td>135-145</td>
</tr>
<tr>
<td>K⁺, mmol/L</td>
<td>4.6</td>
<td>3.5-5.1</td>
</tr>
<tr>
<td>Bicarbonate, mmol/L</td>
<td>18</td>
<td>22-32</td>
</tr>
<tr>
<td>Anion gap, mmol/L</td>
<td>20</td>
<td>10-18</td>
</tr>
<tr>
<td>Osmolality (measured), mosmol/kg</td>
<td>429</td>
<td>278-305</td>
</tr>
<tr>
<td>pH</td>
<td>7.29</td>
<td>7.31-7.45</td>
</tr>
<tr>
<td>FCO₂, mmHg</td>
<td>36.8</td>
<td>35-48</td>
</tr>
<tr>
<td>PO₂, mmHg</td>
<td>102.6</td>
<td>80-104</td>
</tr>
<tr>
<td>Total CO₂, mmol/L</td>
<td>18.6</td>
<td>19-28</td>
</tr>
<tr>
<td>O₂ saturation, %</td>
<td>96.9</td>
<td>95-99.9</td>
</tr>
</tbody>
</table>

Results

On admission, the patient's methanol concentration was estimated to be \(-134\) mmol/L, based on the calculation of the osmolar gap. However, analysis of the serum sample by GC with split-injection revealed a methanol concentration of only 95 mmol/L and no apparent explanation for the EOG of 39 mosmol/kg. No traces of other alcohols were present on the chromatogram, nor had the patient received intravenous solutions of manitol or any other osmotically active compound in the hospital. EOG was even greater in a subsequent sample (EOG 50), but then gradually diminished during treatment with ethanol and hemodialysis.

The evolution of methanol and ethanol concentrations as well as the corresponding osmolar gap and EOG values on the consecutive samples is shown in Fig. 1A.

Although the measured methanol concentrations were reproducible, the results of controls (ethanol/methanol in water) were well within the acceptable ranges, and the measured ethanol levels were not significantly

Bruyne, Dendermonde, Belgium. Concentrations of methanol and ethanol were quantified with an HP 5890 gas chromatograph (GC) equipped with a flame ionization detector (Hewlett-Packard, Brussels, Belgium). We used a 50 m × 0.32 mm (i.d.) fused silica capillary column coated with 1.2-μm CP Sil 5 CB (Chrompack, Antwerp, Belgium). For initial determinations we used a split/splitless injector in the split mode.

We prepared 100 g/L solutions of methanol and ethanol by weighing and making volumetric dilutions; these were used to prepare the methanol and ethanol standard solutions by addition to pooled alcohol-free serum. Sample preparation consisted of adding 200 μL of internal standard solution (3.13 g/L acetonitrile in water) to 100 μL of serum, after which 1 μL was injected into the GC system at a split ratio of 1/60.

We reanalyzed the samples by head-space analysis, using an HP 19396A head-space sampler equipped with a 1-mL loop. Sample preparation for head-space analysis consisted of adding 100 μL of the same internal standard solution to 400 μL of sample. In an oil bath set at 80°C, the samples were incubated for 10 min before injection in the split mode (split ratio, 1/60).

In both GC methods the injector temperature was 250°C and the detector temperature 275°C. The initial oven temperature was increased 30 s after injection from 50°C to 150°C at a rate of 15°C/min, and then maintained for 2 min.

The TDx analyzer (Abbott, Ottignies, Belgium) was used as an alternative procedure for determining serum ethanol concentrations. The rank sum test (two-tailed) was used for comparison of the alcohol concentrations obtained by different methods. \(P < 0.05\) was considered statistically significant.

Calculated osmolality was derived from the following equation, according to Gennari (5):

\[
\text{Osm}_{\text{calc}} = 2\text{Na}^+ \text{ (mmol/L)} + \text{glucose (mmol/L)} + \text{urea (mmol/L)}
\]

Results were compared with those obtained with the formula proposed by Dorwart and Chalmers (6):

\[
\text{Osm}_{\text{calc}} = 1.86 \text{Na}^+ + \text{glucose} + \text{urea} + 9.
\]
different from those obtained with the TDx procedure, we decided to rerun the samples on the same capillary column but using a head-space sampling technique. This time, significantly higher methanol concentrations were found, and the EOG values were no longer excessive (Fig. 1B). Also, the ethanol concentrations measured with both GC methods differed significantly (P <0.05).

Discussion

Although most GC methods for measuring methanol or ethanol (or both) use 1-propanol or 1-butanol as the internal standard, we preferred the use of acetonitrile because alcoholic drinks may contain higher-aliphatic alcohols such as 1-propanol, 2-propanol, 1-butanol, 2-butanol, isobutyl alcohol, 2-methyl-1-butanol, and 3-methyl-1-butanol in sufficient concentration to be detectable in blood or to interfere with the analysis (7). Also, acetonitrile intoxications are less likely to occur than propanol or butanol intoxications. Using the CP Sil 5 CB fused silica capillary column and the described temperature program, we can easily separate methanol, ethanol, 1-propanol, 2-propanol, acetone, 1-butanol, 2-butanol, and acetonitrile.

Initially, a linear response was observed with both GC methods up to concentrations of 4.0 g/L for both ethanol and methanol (87 and 125 mmol/L, respectively). Recoveries typically varied between 96.7% and 98.4% for both alcohols within the range of concentrations varying from 0.50 to 2.0 g/L (ethanol, ~11 to 43.5 mmol/L; methanol, ~15.5 to 62.5 mmol/L), and CVs ranged from 2.1% to 0.6%. Furthermore, water and serum could be used interchangeably, indicating the absence of matrix effects.

The most obvious explanation for the discrepant results we observed for methanol in the described case is discrimination and/or adsorption at the injector site. Discrimination can be quite consistent and cannot be ruled out merely on the basis of reproducibility (8). Previous evaluations of linearity and reproducibility of both our GC methods had revealed similar performance characteristics. However, split/splitless injection of liquid samples of low purity may cause deposition of non-volatile components that gradually accumulate on the vaporization surfaces or on the liner of the injector and then interfere with subsequent injections. When using a head-space sampling technique, the problem of contamination of the injector becomes negligible, because only volatile compounds enter the GC system.

Adsorption effects associated with the direct split/splitless injection could be demonstrated in further experiments, involving repeated injections, exchange of injector liners, and cleaning of the injection port. The degree of adsorption could not always be related to visual contamination of the GC injector. Furthermore, adsorption effects were not always limited to methanol but often also affected the ethanol and acetonitrile peak areas. However, they systematically resulted in lower methanol and often also lower ethanol values.

In the described case, the discrepancies for the two sets of results were most obvious with methanol. This is most probably due to the higher initial methanol concentrations, compared with those for ethanol. Statistical analysis indicated that neither the ethanol results obtained by the direct split/splitless GC method, nor those obtained by head-space analysis, were significantly different from the TDx values, although the differences between the two sets of GC results were significant.

An attentive review of the reports of Meatherall and Krahn (4) and LeGatt and Audette (9) indicates that at least two factors seem to be of major importance in the observation of EOG after ingestion of methanol: the GC method for determining the alcohol concentrations, and the formula for calculating osmolality.

One difference between these studies (4, 9) that has been overlooked in previous discussions is the GC method used. In the cases with abnormally high EOG after methanol ingestion described by Meatherall and Krahn (4), a direct injection technique was used. As with split/splitless injection of liquid samples, direct injection is also prone to contaminate the GC injector or the injector side of the GC column. In contrast, LeGatt and Audette (9) used head-space gas chromatography and reported that, in their experience, the majority of cases of methanol intoxication were not associated with EOG, whereas the few cases that presented with an increased EOG could be explained by other factors.

Another point of difference, which has already been discussed, concerns the formula used for calculation of serum osmolality. Meatherall and Krahn (4) applied the Dorwart and Chalmers formula (6). This formula has been validated in serum samples of patients who had not ingested alcohol (10), where it behaved almost exactly as it had originally been stated, the mean difference from measured osmolality being 1.5 mosmol/kg (SD 5.3 mosmol/kg). With physiological sodium concentrations, however, this formula systematically yields calculated osmolality values 10 to 11 mosmol/kg less than those obtained with the formula proposed by Gennari (5). Consequently, osmolal gap values calculated according to Dorwart and Chalmers will be considerably higher than those calculated by Gennari's formula. On the other hand, using Dorwart and Chalmers' formula, Geller et al. (10) demonstrated that the behavior of ethanol in serum did not conform to that expected from ideal solutions, thus necessitating use of a factor considerably <1.0 to calculate ethanol concentrations (mmol/L) from osmolal gap values. If we assume that methanol in serum also does not behave like an ideal solution, a similar correction factor may be required when calculating methanol concentrations from osmolal gap values. Without correcting for the nonideal behavior of methanol and ethanol, and using the alcohol concentrations as determined by the head-space method, if we apply the Dorwart and Chalmers formula to our own data, the EOG would be +20, +26, and +14 mosmol/kg for the patient's initial, third, and last samples, respectively. However, if we use a correction factor of 0.83 (10) to calculate both ethanol and
methanol concentrations (mmol/L) from osmolal gaps, the EOG would be only −5, −3, and +13 mosmol/kg, respectively.

Thus, although the formula proposed by Gennari probably oversimplifies the calculation of serum osmolality, its application reduces the risk of overestimating alcohol concentrations from osmolal gap values or of finding an EOG.

Various hypotheses have been formulated to explain the EOG phenomenon, including total combined analytical error and underestimation of calculated osmolality. Irrespective of the degree of influence of each of these possible cofactors and considering our experience with the described case, we believe that discrimination and (or) adsorption effects at the GC injector site should first be excluded when searching for the possible cause of an abnormally high EOG after methanol ingestion.

References