Fatal methanol intoxication with different survival times—Morphological findings and postmortem methanol distribution

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Received 13 February 2007; received in revised form 22 May 2008; accepted 25 May 2008
Available online 17 July 2008

Abstract

Three corresponding cases of fatal methanol intoxication with different survival times were investigated ante-mortem and postmortem. Ante-mortem serum methanol concentrations were determined during treatment in hospital for 4 days. Furthermore, postmortem distribution of methanol in various tissues and fluids was measured after autopsy. Morphological and toxicological findings are discussed based on the literature. The morphological findings correlated with the different survival times. The results of the toxicological analyses were partly in keeping with previously published data. Interestingly, very high methanol levels were determined in brain with very low concentrations in femoral venous blood. These results may have implications for postmortem toxicological analysis, brain death diagnosis and organ explanation for transplantation.

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Keywords: Poisoning; Methanol; Acidosis; Survival time; Organ transplantation; Brain death

1. Introduction

Methanol or wood alcohol is used extensively as a solvent in industry, but because of its known toxicity it is not used in household chemicals in higher concentrations in Germany today. However, low methanol concentrations are found in a variety of alcoholic beverages as a result of the conversion of pectin to methanol during alcoholic fermentation. The statutory threshold values in Germany are max. 1000–1350 mg/100 mL pure ethanol. The characteristic occurrence of methanol in different beverages can be useful in forensic cases to assess the amount of post-offence drinking in relation to pre-offence drinking [1].

From the toxicological point of view, methanol levels in commercially available alcoholic drinks are rarely relevant, as the drinks contain sufficient amounts of the antidote ethanol. In contrast, self-made alcoholic beverages or chemicals for hobby purposes, e.g. gasoline for model aircraft often contain high methanol levels that can lead to accidental poisoning [2–6]. Most patients survive, but in some cases, fatal outcome cannot be prevented despite of intensive care, hemodialysis and antidote therapy. For example, in Northern Germany, one out of 10 severe cases of methanol intoxication (1997–2001) were fatal [7]. In a methanol mass poisoning due to illegal spirits in Estonia (September 2001), 68 from 153 patients died (25 in the hospital, 43 outside) [8]. In the period from 1996 to May 2007, 186 cases of methanol exposition (15 severe and 15 life-threatening intoxications) were documented in Northern Germany. Four individuals died of methanol poisoning (three of these cases portrayed in this article) [9].

Methanol is rapidly absorbed into the bloodstream and is then metabolised to formaldehyde and formic acid mainly by the hepatic alcohol dehydrogenase [10]. Toxic effects can mainly be attributed to high formic acid concentrations [11,12]. The slow degradation of formic acid causes accumulation of this toxic acid in human body [13]. Two pathways have been suggested for the formation of formic acid: oxidation to carbon dioxide either through the catalase-peroxidative system or the tetrahydrofolic acid (THF)-dependent one-carbon pool in liver and also retina [11,14].
Thus folic acid can be used to increase formic acid elimination in cases of intoxication.

The selective neurotoxicity of methanol is due to a “histotoxic hypoxia” as a result of inhibition of the cytochrome oxidase complex of the mitochondrial respiratory chain and increased oxidative stress, mainly due to the metabolic end product formic acid [11,15]. It is known that formaldehyde also has cytotoxic effects due to its interaction with electrophilic carbonyl atoms and the amino groups of cell proteins and nucleic acids [16].

Clinical symptoms of methanol intoxication are drowsiness, headache, nausea, vomiting, severe epigastric pain, renal insufficiency, respiratory failure and a central nervous system depression up to coma. Laboratory investigations show a severe anion gap acidosis. Some of the early symptoms like, e.g. impaired speech and coordination can resemble acute ethanol effects. Visual disturbances or permanent visual damage are frequently present in cases with longer survival times [17].

Most of the symptoms occur after an asymptomatic latent period of 12–24 h [3,12]. Radiologic findings typically include cerebral and subarachnoid hemorrhage, edema, bilateral putaminal necrosis and cerebellar cortical lesions [18].

The distribution of methanol in different tissues after absorption is not well understood. As the three cases we investigated in this study have a known time of intake and known survival times, our results contribute to the understanding of methanol tissue distribution in fatal intoxications and the interpretation of toxicological and morphological findings in such cases. Furthermore, the influence of medical treatment on postmortem analysis is discussed.

2. Materials and methods

2.1. Ante-mortem samples

During hospital treatment, 5 mL of preserved blood were sent to our laboratory each day for toxicological analysis.

2.2. Postmortem specimens

Femoral venous and cardiac blood, vitreous humor, urine, liquor, liver tissue and brain tissue were collected at autopsy. Aliquots of these materials (max. 10 g) were immediately placed in 20 mL glass vials covered with rubber caps, sealed and subjected to toxicological analysis without further processing. The samples were stored at 4 °C for max. 14 days until analysis.

2.3. Sample preparation for neuropathology

For neuropathologic examination, the whole cerebrum was fixed in a 4% formalin solution for 6 weeks. Areas of interest were then blocked in paraffin and processed for routine histology according to standard protocols.

2.4. Sample preparation for analysis of methanol levels

From postmortem specimens, 1 mL fluid or 1 g chopped tissue were placed in 20 mL glass vials and 700 mg sodium sulfate was added. Tertiary butanol was used as internal standard and was added in a concentration of 2.44 mg/L.

2.5. Chemicals and reagents

Tertiary butanol, methanol, anhydrous sodium sulfate and distilled water (Lichrosolv) were supplied by Merck Co. (Darmstadt, Germany). Stock solution of t-butanol (100 mg/L) as internal standard was prepared in distilled water and stored at 4 °C.

Medidrug quality controls for congeners alcohol in human serum (BGS S) were purchased from LCG Promochem (Wesel, Germany).

2.6. Instrumentation and chromatographic conditions

Analyses of methanol and ethanol were performed using a headspace gas chromatograph equipped with a flame ionization detector (FID) and a headspace sampler (Clarus 500 GC, PerkinElmer Instruments (Überlingen, Germany)). An Elite 624 capillary column (75 m × 0.53 mm i.d., 3.00-μm film thickness, PerkinElmer) was used for the GC separation of the analytes. Carrier gas was nitrogen, flow 1.13 mL/min, 60 kPa. The operation conditions were as follows: The injector temperature was 170 °C, the detector temperature 280 °C. During the analysis the oven temperature program was: 35 °C for 10 min, ramped to 170 °C during 25 min and 5 min hold at 170 °C. Samples were heated in the head space glass vials for 40 min at 60 °C for equilibration before injection.

2.7. Data processing

Analyte responses were integrated with automatic quantification software (Turbochrom, PerkinElmer) and quantification was carried out based on the peak area responses of the analyte with respect to the internal standard via a daily calibration curve with eight calibration points. In cases of higher concentrations of methanol in the first analysis (>5000 mg/L), a new sample was diluted 1 + 1 or 1 + 9 with distilled water and the analysis was repeated.

2.8. Validation of the analytical method

The analytical method was validated for human serum according to the guidelines of the GTFCh (Society of Toxicological and Forensic Chemistry, www.gtfch.org). The limit of determination (LOD) for methanol in blood is 15 mg/L, the limit of Quantification (LOQ) is 53 mg/L. Linearity is up to 5000 mg/L ($R^2 = 0.996$).

3. Case report and results

Case 1: A 39-year-old man was admitted to hospital in a deep coma. Because of his bad condition, brain death diagnosis and subsequent organ donation was considered. According to the “Guidelines for Determining Brain Death” published by the German Federal Council of Doctors [19], a toxicological investigation was assigned prior to brain death determination in order to exclude intoxication or the influence of narcotising substances. Because of the severe metabolic acidosis, an analysis for glycol, metformin and methanol was carried out in addition to the routine general unknown-analysis for centrally depressant substances. A toxic methanol serum concentration of 5400 mg/L was detected in this analysis (Table 1), with negative results for all other substances. Treatment with ethanol and folic acid, bicarbonate and hemodialysis was started immediately after the detection of methanol poisoning (day 2 of hospitalisation).

Case 2: A 34-year-old man was admitted to the same hospital. As in case 1, the patient was presenting with coma and severe acidosis. A strong suspicion of methanol intoxication was raised, and a blood sample was sent to our laboratory for toxicological investigation on the following day. As in case 1,
we found a toxic serum methanol level of 7400 mg/L (Table 1). Treatment was started immediately as described in the first case (day 2 of hospitalisation).

Despite intensive care and specific therapy, the patients died 4 and 3 days after admission, respectively.

**Case 3:** A 44-year-old man was found dead in a garden shed 3 days after the first case had occurred.

Police investigations revealed that all three men had known each other and had celebrated together in the garden shed. All men were known to be alcoholics. At first, the source of the methanol was unknown. Further investigations solved this mystery: one of the men had found a canister of thinner for flower paint, which contained pure methanol, on the grounds of a former hotel. The three men had decanted the solvent into empty vodka and lemonade bottles, which were found in the shed and surroundings, and drank it after dilution with water and addition of salt.

The bodies of all three victims were subjected to medico-legal autopsy at the Department of Legal Medicine, University of Hamburg, Germany, for medico-legal investigation.

At autopsy, brain and pulmonary edema were present in all cases. Gross neuropathologic examination revealed hemorrhage of the basal ganglia in cases 1 and 2 (Fig. 1) and necrosis of the basal ganglia in case 3 (Fig. 2).

At histology, case 3 only exhibited mild capillary congestion and hyperemia in the lateral putamen, whereas in cases 1 and 2, hemorrhagic necrosis of the putamen was found. In addition, cases 1 and 2 showed hemorrhages in the tissue surrounding the optic nerve, but no signs of demyelinisation of the nerve itself. Other major organ changes were not found at gross inspection or histology.

An overview of the postmortem toxicological analyses is given in Table 2.

In the first two cases with longer survival times, the concentrations of methanol in nearly all fluids and tissues were very low due to hemodialysis. Brain stem methanol levels were very high in both cases, with concentrations of 738 and

<table>
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<th>Day</th>
<th>Specimen</th>
<th>Methanol mg/L</th>
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<tbody>
<tr>
<td>1</td>
<td>Serum</td>
<td>5400</td>
<td>300</td>
</tr>
<tr>
<td>2</td>
<td>Serum</td>
<td>420</td>
<td>300</td>
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<tr>
<td>3</td>
<td>Serum</td>
<td>190</td>
<td>9</td>
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<tr>
<td>4</td>
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<td>160</td>
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<td></td>
<td>Femoral blood</td>
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<tr>
<td></td>
<td>Femoral blood</td>
<td>220</td>
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1008 mg/kg, respectively. In the patient who died without any medical treatment, the methanol was equally distributed with levels of approx. 2000 mg/L in all investigated tissues and fluids.

Significant differences in methanol concentrations of cardiac and femoral blood were not found: 5/5, 254/228, and 2130/2074 mg/L, respectively. Methanol levels in vitreous humor and femoral venous blood were in the same range with levels of 8/5, 201/228 and 2122/2074 mg/L (Table 2).

4. Discussion

The initial ante-mortem serum methanol concentrations of 5400 and 7400 mg/L (see Table 1) are high above most of those previously published (360–3000 mg/L) [3–6,20–23]. Only two cases with higher serum methanol levels have been reported: Wu et al. determined 11240 mg/L in a 5-week-old infant, suffering from only moderate metabolic acidosis due to a low hepatic ADH activity in young infants [2]. Wu-Chen et al. reported a case of fatal methanol ingestion with 5380 mg/L serum methanol on admission to the emergency room [24]. Apart from this, López-Navidad et al. reported of successful organ-transplantation from donors who died of methanol intoxication, and has already been carried out successfully in some hospitals [25,29,30].

The calculated amounts of thinner with 100% methanol that must have been ingested in our cases 1 and 2 as determined by Widmark’s formula were approximately 350 and 450 mL, respectively.

The postmortem methanol concentrations determined in case 3, the man who died without treatment, were within a range expected after intoxication. Distribution in all organs compared well with those reported for other methanol fatalities [13,20,24].

Surprisingly, in cases 1 and 2, the postmortem methanol concentrations in brain tissue were much higher than the blood levels (738 mg/kg vs. 5 mg/L and 1008 mg/kg vs. 228 mg/L) and other postmortem specimens (see Table 2). In previous cases, much smaller differences have been reported between the different samples. In a case of a patient who was treated with ethanol, bicarbonate and hemodialysis and expired 40 h after admission, brain methanol concentration was reported at 1590 mg/kg vs. 1420 mg/L in blood [24]. Ferrari et al. reported average methanol concentrations of 1980 mg/kg and 1750 mg/L in brain or blood samples [13]. In other cases, brain tissue was not available for determination [20].

One possible explanation for the high brain methanol concentrations compared to other tissues could be that methanol as an organic solvent accumulates in the lipophilic brain tissue. This phenomenon is especially pronounced with high lipophilic molecules for example organic solvents like gasoline. However, this has never been suggested for methanol—most likely because, like ethanol, it is known to be distributed depending on the water content of the respective tissue ($V_d = 0.6–0.7$ L/kg) [10,26].

Another perhaps more likely explanation could be that methanol could not be eliminated from the brain because of the disturbed redistribution by the inhibited blood flow due to brain edema. In our cases, both men developed brain edema during the time in hospital, which has been reported previously in cases of methanol poisoning [27]. For the metabolism of alcohols in brain, catalase and cytochrome P450 2E1 are more important than alcohol dehydrogenases [28]. It is yet unknown which kind of toxic effects will develop at a high concentration of methanol only in the brain. CNS-depressing effects due to its properties as an organic solvent and additional toxic effects due to its metabolism to formaldehyde and formic acid and the consecutively increased oxidative stress are possible mechanisms.

The phenomenon of a possible accumulation of methanol in the brain is especially important as there are several publications reporting that organ transplantation is possible from donors who died of methanol intoxication, and has already been carried out successfully in some hospitals [25,29,30].

The morphological findings in our three cases were in line with those previously described [3,5,6,10,31–33]. The severity of these neuropathologic changes correlated well with the survival time.

5. Conclusion

Our results demonstrate once more that hemodialysis can effectively reduce toxic blood methanol concentrations [12,21,22]. It could also be shown, however, that the determination of blood levels does not reliably reflect brain methanol concentrations. In fact, brain concentrations can be several times higher than blood levels. This should always be considered when treating patients for methanol intoxication. If a patient presents in a coma, the narcotic properties of methanol should be kept in mind before starting brain death diagnosis [10,26].

In contrast to Ferrari et al. [13], we found that after ingestion of very large amounts of methanol, methanol and not only formic acid could be used as a marker for methanol intoxication several days after ingestion (see Table 1). On the basis of our results we recommend that if fatal methanol intoxication is suspected, brain methanol analysis should be performed after autopsy in addition to blood analysis to avoid that cases with longer survival times escape detection.
References


