URINARY EXCRETION OF METHANOL AND 5-HYDROXYTRYPTOPHOL AS BIOCHEMICAL MARKERS OF RECENT DRINKING IN THE HANGOVER STATE

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Abstract — Twenty healthy social drinkers (9 women and 11 men) drank either 50 g of ethanol (mean intake 0.75 g/kg) or 80 g (mean 1.07 g/kg) according to choice as white wine or export beer in the evening over 2 h with a meal. After the end of drinking, at bedtime, in the following morning after waking-up, and on two further occasions during the morning and early afternoon, breath-alcohol tests were performed and samples of urine were collected for analysis of ethanol and methanol and the 5-hydroxytryptophol (5-HTOL) to 5-hydroxyindol-3-ylacetic acid (5-HIAA) ratio. The participants were also asked to quantify the intensity of hangover symptoms (headache, nausea, anxiety, drowsiness, fatigue, muscle aches, vertigo) on a scale from 0 (no symptoms) to 5 (severe symptoms). The first morning urine void collected 6-11 h after bedtime as a rule contained measurable amounts of ethanol, being 0.09 ± 0.03 g/l (mean ± SD) after 50 g and 0.38 ± 0.1 g/l after 80 g ethanol. The corresponding breath-alcohol concentrations were zero, except for three individuals who registered 0.01-0.09 g/l. Ethanol was not measurable in urine samples collected later in the morning and early afternoon. The peak urinary methanol occurred in the first morning void, when the mean concentration after 80 g ethanol was ~6-fold higher than pre-drinking values. This compares with a ~50-fold increase for the 5-HTOL/5-HIAA ratio in the first morning void. Both methanol and the 5-HTOL/5-HIAA ratio remained elevated above pre-drinking baseline values in the second and sometimes even the third morning voids. Most subjects experienced only mild hangover symptoms after drinking 50 g ethanol (mean score 2.4 ± 2.6), but the scores were significantly higher after drinking 80 g (7.8 ± 7.1). The most common symptoms were headache, drowsiness, and fatigue. A highly significant correlation (r = 0.62-0.75, P < 0.01) was found between the presence of headache, nausea, and vertigo and the urinary methanol concentration in the first and second morning voids, whereas 5-HTOL/5-HIAA correlated with headache and nausea. These results show that analysing urinary methanol and 5-HTOL furnishes a way to disclose recent drinking after alcohol has no longer been measurable by conventional breath-alcohol tests for at least 5-10 h. The results also support the notion that methanol may be an important factor in the aetiology of hangover.

INTRODUCTION

Besides the acute effects of ethanol on a person’s performance and behaviour, common after-effects of heavy drinking, referred to as the hangover state, include headache, nausea, and vomiting. Furthermore, less than a quarter of people with hangover report more psychiatric symptoms like depression and nervousness (Smith and Barnes, 1983). What is less common knowledge and poorly studied, however, is impairment of mental and body functioning, such as fatigue, attention deficit, and diminished psychomotor skills (Kelly et al., 1970; Seppälä et al., 1976; Lemon et al., 1993; Ames et al., 1997). It seems likely that many accidents on the roads and in the workplace might be attributed to decreased attention and slower reaction time associated with the residual effects of an evening’s heavy drinking (Karvinen et al., 1962; Törnros and Laurell, 1991; Roehrs et al., 1994).

Although neither dose–response relationships nor the prevalence of hangover have been clearly established, experience has shown that a person’s blood-alcohol concentration (BAC) should exceed approximately 1.0 g/l (100 mg/dl) during the acute
phase of intoxication (Collins and Chiles, 1980; Smith and Barnes, 1983). Hangover starts to develop when BAC decreases again and is approaching zero. However, the intensity and duration of hangover show large intra- and interindividual variations and the reduced performance in some people might persist for many hours after all ethanol has been cleared from the body (Goldberg, 1961). The mechanism whereby acute ethanol intoxication causes hangover remains an unsolved problem. In addition to dehydration and a disturbed acid-base balance, alcoholic beverages rich in congeners seem to cause more severe hangover symptoms than equivalent doses of pure ethanol (Pawan, 1973; Jones, 1987).

Low concentrations of ethanol and methanol (0.5–1.0 mg/l) can be determined in body fluids even without drinking alcohol beverages (Majchrowicz and Mendelson, 1971). These endogenous alcohols are oxidized in the liver to their respective aldehydes by alcohol dehydrogenase (ADH, EC 1.1.1.1) and the affinity of this enzyme is roughly 10 times higher for ethanol as its substrate compared with methanol. This means that the concentrations of endogenous methanol in blood and other body fluids increase during the time people drink alcoholic beverages because ADH is fully engaged in the metabolism of ethanol (Majchrowicz and Mendelson, 1971). Methanol occurs as a congener in alcoholic beverages, which also contributes to the raised concentrations observed in body fluids after a drinking spree (Majchrowicz and Mendelson, 1971). The elimination half-life of methanol in humans is between 2 and 4 h (Jones, 1987; Haffner et al., 1992) so that methanol can be detected in blood, breath, and urine for long after ethanol has returned to its endogenous concentration (Jones, 1986).

Acetaldehyde is the primary metabolite of ethanol oxidation and this noxious substance is rapidly converted to acetate through the action of aldehyde dehydrogenase (ALDH). However, ALDH is also involved in the oxidation of biogenic aldehydes, such as those formed during catabolism of dopamine, noradrenaline, and serotonin (5-hydroxytryptamine). The urinary excretion products of serotonin metabolism are 5-hydroxyindol-3-ylacetic acid (5-HIAA) and 5-hydroxytryptophol (5-HTOL), and, without drinking any alcohol, the ratio of 5-HTOL/5-HIAA is normally very low (<0.01) (Helander et al., 1993). When acetaldehyde derived from ethanol competes with the biogenic aldehyde formed from serotonin for available ALDH, there is a switch in the metabolic pathway and the 5-HTOL/5-HIAA ratio in urine increases appreciably and does not recover to baseline levels until several hours after ethanol has been cleared from the blood (Helander et al., 1993).

During rehabilitation of alcoholics and others who are forbidden to drink alcohol as part of their treatment, a sensitive and specific test of recent drinking is desired. Breath-ethanol testing is not sufficiently sensitive to control whether a patient has remained abstinent, because many people can regulate their intake of alcohol. However, owing to the time-lag in urinary excretion of methanol and the 5-HTOL/5-HIAA ratio compared with ethanol, these tests furnish a way to disclose recent drinking even after ethanol has been cleared from the body (Helander et al., 1996; Jones and Helander, 1996), and this coincides in time roughly with the appearance of hangover symptoms. During medicolegal investigations into the likely causes of serious accidents on the road or in the workplace, it is obviously important to establish whether or not recent heavy drinking leading to hangover was a factor involved (Hagan and Helander, 1997). This might be relevant in civil and criminal litigation when responsibility for the accident and insurance claims are made (Ames et al., 1997).

We conducted a controlled drinking experiment to investigate the usefulness of methanol and 5-HTOL/5-HIAA as biochemical markers of acute alcohol ingestion, and to test how long after the end of drinking these markers remain valid compared with measuring ethanol in blood, breath and urine. Also in this study, an attempt was made to quantify the intensity of hangover symptoms during the following morning and to relate them to urinary levels of methanol and the 5-HTOL/5-HIAA ratio.

**MATERIALS AND METHODS**

**Subjects and experimental design**

Twenty healthy social drinkers (9 women and 11 men with no history of excessive drinking; employees at the University Hospital, Linköping)
with a mean age of 39 ± 9.4 years (SD) and mean body weight of 72 ± 11.6 kg participated in this study, as paid volunteers. No intake of alcohol was allowed for 48 h before starting the experiment. They drank either 50 or 80 g of ethanol according to choice as white wine (11% ethanol, v/v) or export beer (5.2%, v/v). The alcohol was consumed in the evening over a period of 2 h with a meal in an attempt to mimic social drinking conditions (no further intake of alcohol was allowed during the experiment). Fifteen minutes after the end of drinking, the breath-alcohol concentration (BrAC) was measured with an Alcolmeter S-D2 and the results were reported as equivalent BAC. Thereafter, specimens of urine (10 ml) were collected in plastic tubes containing 100 mg sodium fluoride as preservative. Another BrAC test was made and further specimens of urine collected just before bedtime. The BrAC was also measured in the morning after arising from bed when samples of urine were also collected. Finally, breath and urine samples were taken on two further occasions during the morning and early afternoon. The times for collecting the consecutive samples were not exactly the same for each subject. Control specimens of urine were either collected before ingestion of alcohol began or on a separate occasion when the subjects had abstained from drinking alcohol for at least 2 days.

In the morning after alcohol intake, the participants were asked to quantify the intensity of seven hangover symptoms on a scale from 0–5, where 0 indicated no symptoms and 5 the most severe symptoms. The hangover symptoms included were headache, nausea, anxiety, drowsiness, fatigue, muscle aches, and vertigo.

**Measurements**

The urinary concentrations of ethanol (UAC) and methanol (UMC) were determined by headspace gas chromatography as reported in more detail elsewhere (Jones and Löwinger, 1988; Jones and Schuberth, 1989). For the analysis of ethanol, the urine was diluted 1:10 with n-propanol (0.8 g/l) as an internal standard and the peak height ratios of ethanol/n-propanol were used for quantitative analysis by comparison with the response from analysis of known strength alcohol standards (0.50, 1.0, and 1.5 g/l). The limit of quantification of the method is 0.01 g/l and the within-run precision expressed as coefficient of variation is 1% at a mean urine-ethanol concentration of 1.0 g/l. For the analysis of methanol, a salting-out procedure was used to improve the analytical sensitivity. This entailed adding the aliquot of urine (0.5 ml) to a headspace vial containing 1.2 g sodium chloride. The vials (22 ml volume) were immediately made air-tight with crimped-on rubber septums before being analysed. The precision (CV) of analysing methanol in urine by this method was 3.4% at a mean concentration of 2.3 mg/l.

The concentrations of 5-HTOL and 5-HIAA in urine were determined by gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC), respectively. For quantification of 5-HIAA, a urine specimen was spiked with an internal standard and a small volume was injected directly into the HPLC system (Helander et al., 1991), whereas for 5-HTOL, which is excreted mainly in conjugated form, enzymatic hydrolysis was done prior to analysis by GC-MS (Beck et al., 1982). Both 5-HIAA and 5-HTOL are relatively stable in urine samples during handling and storage. To compensate for variations in urine flow rate and also dietary intake of serotonin, which are factors that might influence results, the ratio of 5-HTOL/5-HIAA was calculated (Helander et al., 1992). The reference limit used in clinical practice to indicate an abnormal 5-HTOL/5-HIAA ratio has been set at >15 pmol/nmol (Helander et al., 1994).

**Statistics**

The concentrations of methanol and the 5-HTOL/5-HIAA ratio in the pre-drinking specimens of urine were compared with results for samples collected after drinking by Student's t-test for paired observations. Comparison of hangover symptoms was performed using the t-test for unpaired observations, and correlations were evaluated by Pearson's test.

**RESULTS**

Figure 1 shows the concentrations of ethanol, methanol, and the 5-HTOL/5-HIAA ratio in urine samples collected before drinking and on five occasions after intake of either 50 g (8 women and 3 men) or 80 g (1 woman and 8 men) of ethanol. The first morning void was collected between 6–11 h after bedtime (05:45–10:30), and at that
Fig. 1. Urinary concentrations of ethanol and methanol and ratio of 5-hydroxytryptophol to 5-hydroxyindol-3-ylacetic acid (5-HTOL/5-HIAA) in healthy subjects before and at various times after oral ethanol consumption.

Urinary concentrations of (a) ethanol and (b) methanol, and (c) the 5-HTOL/5-HIAA ratio were measured after healthy subjects drank 50 g (n = 11) or 80 g (n = 9) ethanol in the evening over 2 h with a meal. The first urine void was collected before starting drinking, the second after the end of drinking, the third void just before bedtime, the fourth in the morning immediately after arising from bed (between 6–11 h after bedtime (05:45–10:30)), and the fifth and sixth voids were obtained later in that morning or early afternoon (collected 1.5–6 h (09:30–12:45) and 3–9 h (12:10–15:30), respectively, after the first morning void).

The results are mean values ± SD.
Table 1. Differences between the concentration of methanol and the 5-HTOL/5-HIAA ratio in pre-drinking specimens of urine and voids 4–6 made in the morning and early afternoon after volunteers drank 50 or 80 g ethanol the previous evening

<table>
<thead>
<tr>
<th>Material analysed</th>
<th>Ethanol dose (g)</th>
<th>Pre-drinking values</th>
<th>1st morning void</th>
<th>2nd morning void</th>
<th>3rd morning void</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol (mg/l)</td>
<td>50</td>
<td>0.77 ± 0.05</td>
<td>3.06 ± 0.40**</td>
<td>0.73 ± 0.16**</td>
<td>0.26 ± 0.09*</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.03 ± 0.11</td>
<td>5.06 ± 0.55***</td>
<td>2.28 ± 0.67**</td>
<td>0.49 ± 0.32</td>
</tr>
<tr>
<td>5-HTOL/5-HIAA (pmol/nmol)</td>
<td>50</td>
<td>6.36 ± 0.52</td>
<td>134 ± 28.7***</td>
<td>4.64 ± 1.23**</td>
<td>1.54 ± 0.41*</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>6.80 ± 0.85</td>
<td>296 ± 47.3***</td>
<td>35.0 ± 8.41**</td>
<td>6.22 ± 2.26*</td>
</tr>
</tbody>
</table>

* Collected in the morning between 6–11 h after bedtime (05:45–10:30).  
* Collected 1.5–6 h after the first morning void (09:30–12:45).  
* Collected 3–9 h after the first morning void (12:10–15:30). Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001 (paired t-test).

0–7) of ethanol. Two of the participants (1 woman and 1 man) were excluded from these calculations, because of severe illness during the evening of the experiment (suspected food poisoning). The most common symptoms reported were headache and drowsiness followed by fatigue. In the first morning void, the UMC was significantly correlated with the hangover symptoms headache, nausea, and vertigo (r = 0.62–0.67, P < 0.01), and to a lesser extent with fatigue, muscle aches and drowsiness (r = 0.49–0.59, P < 0.05). In the second morning void, the UMC was significantly correlated with headache and nausea (r = 0.75–0.76, P < 0.001) and to a lesser extent with vertigo (r = 0.65, P < 0.01). The 5-HTOL/5-HIAA ratio was significantly correlated with headache and nausea in both the first (r = 0.50–0.53, P < 0.05) and second (r = 0.66–0.67, P < 0.01) morning voids. The feelings of anxiety displayed very low correlations with methanol and the 5-HTOL/5-HIAA ratio in the morning urine.

DISCUSSION

It is common knowledge that people do not function normally when they are suffering from hangover and this can be demonstrated when skilled tasks must be performed (Kelly et al., 1970; Seppälä et al., 1976; Yesavage and Leirer,

Table 2. Intensity and frequency of hangover symptoms in the morning after healthy subjects (n = 18) drank 50 or 80 g ethanol over 2 h the previous evening with a meal

<table>
<thead>
<tr>
<th>Hangover symptom</th>
<th>Number of subjects reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 g</td>
</tr>
<tr>
<td>Headache</td>
<td>5</td>
</tr>
<tr>
<td>Nausea</td>
<td>8</td>
</tr>
<tr>
<td>Anxiety</td>
<td>7</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>5</td>
</tr>
<tr>
<td>Fatigue</td>
<td>8</td>
</tr>
<tr>
<td>Muscle aches</td>
<td>10</td>
</tr>
<tr>
<td>Vertigo</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
</tr>
</tbody>
</table>

* Two participants were excluded from these calculations because of illness during the evening of the experiment (suspected food poisoning).

* The score range is 0-5 where 0 indicates no symptoms and 5 severe symptoms.
concentrations of methanol and the 5-HTOL/5-HIAA ratio could often be distinguished from the pre-drinking values after drinking 50 or 80 g ethanol, the last specimen of urine would not be of much practical use without knowledge of a person's pre-drinking levels. This information is not generally available.

We are advocating the analysis of methanol in urine as a marker of acute intake of alcohol and this test is sufficiently sensitive to disclose ingestion of alcohol even after alcohol has been cleared from the body. However, finding an abnormally high concentration of methanol in body fluids might also indicate chronic alcohol intake (Roine et al., 1989; Iffland, 1996), because during long-term heavy drinking, methanol accumulates gradually to reach fairly high concentrations and complete clearance might require several days. If the concentration is above 10 mg/l in serum this is considered a marker of heavy continuous drinking for days or weeks (Iffland, 1996). It should be pointed out that the analysis of methanol alone is not completely specific as a test for alcohol consumption, because eating fresh fruits or drinking large volumes of fruit juice containing traces of free methanol or methyl esters can partly account for the urinary excretion of this alcohol (Gruner and Bilzer, 1983; Lindinger et al., 1994). The artificial sweetener aspartame can be converted in the body into methanol (Davoll et al., 1986). Accordingly, an elevated concentration of methanol in urine needs to be supported by other evidence or a more specific marker of alcohol consumption, such as an elevated 5-HTOL/5-HIAA ratio. The baseline value of the 5-HTOL/5-HIAA ratio is not increased after prolonged heavy drinking and this test can therefore be used to reveal recent drinking (within the past ~24 h) in both social and chronic consumers (Voltaire-Carlsson et al., 1993; Helander et al., 1994).

Since the peak of methanol excretion in the urine coincides with the time when hangover symptoms are worst, it has been suggested that methanol, or metabolites thereof, play a role in the development of hangover (Jones, 1986; Calder, 1997). This is further supported by observations that alcoholic beverages rich in methanol, such as brandy, whisky, and red wine, cause more severe hangover symptoms than equivalent doses of pure ethanol (Chapman, 1970; Jones, 1987). The findings in this study of a correlation between
the methanol level and severity of hangover symptoms support this notion, but it should be pointed out that the development of hangover is probably a combination of physical and biological factors together with psychological and hereditary factors resulting in a large inter-individual variability (Smith and Barnes, 1983).

In conclusion, analysing methanol and the 5-HTOL/5-HIAA ratio in urine furnishes a way to disclose recent drinking after alcohol is no longer measurable by conventional breath-alcohol tests for at least 5–10 h. Although 5-HTOL/5-HIAA is a more specific marker for recent drinking, methanol can give further support for 5-HTOL/5-HIAA findings by a different mechanism. There will certainly be applications as relapse markers during rehabilitation of alcoholics and drug addicts as well as convicted drunk drivers who must refrain from drinking as a part of their treatment. These tests might also have useful applications when investigating serious accidents on the roads or in the workplace to disclose whether a person was engaged in heavy drinking the night before the accident (hangover effect). Finding an elevated urinary concentration of methanol and an elevated 5-HTOL/5-HIAA ratio provides convincing evidence of recent drinking, even if ethanol cannot be determined in body fluids. Because a person’s pre-drinking levels of urinary methanol and the 5-HTOL/5-HIAA ratio are not usually known, threshold values of 2.0 mg/l for methanol (according to this study) and 15 pmol/nmol for 5-HTOL/5-HIAA (Helander et al., 1994) seem to be reasonable values for clinical and forensic purposes when evaluating random supplies of urine.

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