5-Hydroxytryptophol (5HTOL), a New Sensitive Urinary Test of Recent Alcohol Consumption

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INTRODUCTION

The traditional method to assess whether a person has recently been drinking is by analysis of breath, blood or urine samples for the presence of alcohol (ethanol). However, the usefulness of these assays is limited by the time course of alcohol in the body. Because ethanol is rapidly metabolised and eliminated from the body, a method to confirm recent drinking even after ethanol has been cleared would have considerable importance. Assay of urinary 5-hydroxytryptophol (5HTOL), a metabolite of serotonin (5-hydroxytryptamine), has shown promise as a way to prolong the detection window of recent alcohol use by several hours compared to measuring ethanol in body fluids or breath (Helander et al., 1994a). This new test has already proven useful, for example to monitor abstinence in alcohol dependent subjects treated as outpatients (Voltaire Carlsson et al., 1993), and to distinguish ingested from microbially formed ethanol in forensic post-mortem specimens (Helander et al., 1992a, 1995b).

METABOLIC INTERACTION BETWEEN ETHANOL AND SEROTONIN

During metabolism of ethanol, serotonin metabolism shifts from production of 5-hydroxyindoleacetic acid (5HIAA) toward formation of 5HTOL (Davis et al., 1967). Urinary 5HTOL concentrations are normally less than 1% of 5HIAA levels, but after alcohol ingestion the metabolic formation of 5HTOL increases dramatically in a dose-dependent manner at the expense of 5HIAA production. After heavy drinking, the concentration of 5HTOL may even exceed that of 5HIAA (Helander et al., 1993). This change follows because ethanol and serotonin share the same catabolic enzymes. Experiments with liver homogenates suggest that the metabolic shift occurs because of competitive inhibition of aldehyde dehydrogenase.
(ALDH) by acetaldehyde derived from oxidation of ethanol (Lahti and Majchrowicz, 1974). Furthermore, during the metabolism of ethanol and acetaldehyde, the reduced coenzyme NADH is present in excess, and this change in redox state promotes formation of 5HTOL via the alcohol dehydrogenase (ADH) pathway (Feldstein and Williamson, 1968). More importantly, however, serotonin metabolism will not normalise until several hours after ethanol is no longer measurable in body fluids or breath (Helander et al., 1993) (Fig. 1). On the basis of this time delay, an elevated urinary concentration of 5HTOL was suggested as a marker to disclose relapse drinking in connection with rehabilitation of alcohol dependent subjects in an outpatient setting (Voltaire et al., 1992).

Fig. 1: Time course of concentrations of ethanol in plasma (P) and urine (U), and the urinary 5HTOL/5HIAA ratio, in samples from 10 alcohol dependent subjects during detoxification (from Helander et al., 1996). The cut-off level used to distinguish between normal and elevated 5HTOL/5HIAA ratios is 15 pmol/nmol. The results are mean values.
OPTIMISING THE USE OF URINARY 5HTOL TO DETECT RECENT DRINKING

To improve the performance of the 5HTOL test, the results should be normalised for urine dilution. This can be done by relating the results to creatinine content. However, experiments with healthy subjects demonstrated that using the ratio of 5HTOL to 5HIAA is preferred since it will compensate for variations caused by urine dilution as well as dietary intake of serotonin (Helander et al., 1992b). For example, ingestion of bananas, a common source of serotonin, produce a distinct peak in the 5HTOL/creatinine ratio but not in 5HTOL/5HIAA. The cut-off level to distinguish between normal and elevated 5HTOL/5HIAA ratios has been set at 15 pmol/nmol (1.5%). This value was derived from analysing specimens from >140 abstaining subjects for whom the ratio was always less than 15 pmol/nmol (Helander et al., 1994b).

Compared to the 5HTOL/5HIAA ratio, the sensitivity and specificity of using the 5HTOL/creatinine ratio or the urinary 5HTOL concentration alone were lower (Table 1). These results, which are based on urine specimens from >500 alcohol dependent patients and >100 controls, show that 5HTOL expressed as a ratio to 5HIAA provides the most sensitive measure of recent alcohol use.

<table>
<thead>
<tr>
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<th>5HTOL/5HIAA* (*&gt;15 pmol/nmol)</th>
<th>5HTOL/creatinine (*&gt;35 nmol/nmol)</th>
<th>5HTOL concentration (*&gt;500 nmol/L)</th>
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<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>100</td>
<td>88</td>
<td>79</td>
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<tr>
<td>Specificity (%)</td>
<td>100</td>
<td>96</td>
<td>91</td>
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* The 5HTOL/5HIAA ratio was used as gold standard (i.e., as being both 100% sensitive and specific). The cut-off levels are given in parenthesis.

ANALYTICAL PROCEDURES

5HTOL and 5HIAA are presently assayed using chromatographic techniques. For quantitation of 5HIAA, crude urine samples are spiked with an internal standard and a small volume in-jected directly into a HPLC system (Helander et al., 1991). 5HTOL, which is
excreted mainly as the glucuronic acid conjugate (Helander et al., 1995a), is liberated from the conjugated form by enzymatic hydrolysis prior to analysis by gas chromatography-mass spectrometry (GC-MS) (Beck et al., 1982; Voltaire et al., 1992). The advantage of this method is its high reliability, although the GC-MS technique is time consuming and therefore less suitable for screening purposes. However, an immunochemical assay for the 5HTOL-glucuronide in urine is presently being developed, and such a direct assay might facilitate the routine clinical use of 5HTOL as biochemical marker of recent alcohol consumption.

SPECIFICITY AND SENSITIVITY OF THE 5HTOL/5HIAA RATIO TEST

The urinary 5HTOL/5HIAA ratio remains stable both within-days and between-days during periods of abstinence in social and heavy drinkers. Moreover, both serotonin metabolites are stable during transport and storage of urine specimens. Gender or genetic variations in ADH and ALDH isozyme pattern do not influence the 5HTOL/5HIAA basal value (Helander et al., 1994b, 1996). Marked changes in the turnover of serotonin (oral loading with serotonin; dietary depletion of tryptophan) alter the steady state concentrations of 5HTOL and 5HIAA, but their relative proportion (5HTOL/5HIAA ratio) remains unchanged (Helander et al., 1992c; unpublished observations). Consequently, commonly used drugs such as monoamine oxidase inhibitors and serotonin reuptake inhibitors are not likely to interfere with the reliability of the 5HTOL test. Apart from alcohol ingestion, elevated 5HTOL/5HIAA ratios have been reported only in patients on medication with the ALDH inhibitors disulfiram (Antabuse) or calcium carbimide (cyanamide) (Beck et al., 1995), but the changes in ratio observed were less than after drinking alcohol and highly variable between subjects. However, during disulfiram maintenance treatment, the continuous inhibition of ALDH produces a new higher 5HTOL to 5HIAA steady state level and relapse to drinking will still lead to increased 5HTOL/5HIAA ratios (Helander, unpublished observations).

In an experiment where the first urinary void in the morning was sampled daily from eight social drinkers over a period of about one month, a significant correlation between amount of alcohol consumed and the 5HTOL/5HIAA ratio was observed (Helander et al., 1996). Assay of the 5HTOL/5HIAA ratio identified on the average 73% of all drinking occasions the previous evening or late afternoon exceeding 7 g of alcohol (range 3-98 g/day according to self-reported drinking protocols), as compared with 22% by analysis of ethanol (>200
μmol/L). On some occasions when relatively large amounts of alcohol had been consumed, elevated 5HTOL/5HIAA ratios occurred also in the second and sometimes even the third urinary voids collected during the morning and early afternoon.

**CLINICAL AND FORENSIC APPLICATIONS OF THE 5HTOL/5HIAA RATIO TEST**

There are obvious applications for the 5HTOL/5HIAA ratio as a relapse marker in connection with rehabilitation of alcohol dependent subjects as well as drunk drivers who must refrain from drinking. In outpatient settings, 5HTOL testing revealed single relapses which were otherwise not detected, such as by self-reports or conventional markers of excessive drinking (Voltaire Carlsson et al., 1993). Furthermore, 5HTOL can be utilised as a biochemical complement to self-reported alcohol consumption when new treatment models and new markers of alcohol misuse are being evaluated. In forensic medicine, the 5HTOL/5HIAA ratio has already been used to detect artifactual formation of ethanol, for example to distinguish ingested from microbially formed ethanol in post-mortem specimens (Helander et al., 1992a, 1995b). Other potential applications of 5HTOL include documentation of previous heavy drinking in workplace testing or when accidents are being investigated (possible impaired performance due to hangover effect) (Jones and Helander, 1996; Hagan and Helander, 1997).

**REFERENCES**


Jones AW and Helander A (1996) Disclosing recent drinking after alcohol has been cleared from the body. *J Anal Toxicol* 20, 141-142.

