Serum/Whole Blood Concentration Ratio for Ethylglucuronide and Ethyl Sulfate

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Abstract

Serum/blood (S/B) concentration ratios for ethyl glucuronide (EtG) and ethyl sulfate (EtS) are missing from the literature, and the aim of this study was to determine these ratios in samples from patients at admission to an alcohol rehabilitation clinic. Two blood samples were collected simultaneously, and EtG and EtS were analyzed in whole blood and serum, respectively, using a liquid chromatography–mass spectrometry method. Separate calibration standards were prepared in both whole blood and serum for the calculation of whole blood and serum concentrations, respectively. Thirteen pairs of serum and whole blood were analyzed. The median S/B value for EtG was 1.69, and the range was 1.33–1.90. For EtS, the median S/B ratio was 1.30, and the range was 1.08–1.47. The S/B ratio was significantly lower for EtS than for EtG (p < 0.001). The higher concentrations of EtG and EtS in serum than in whole blood have to be considered when whole blood results obtained from forensic toxicology are compared to serum or plasma results from clinical laboratories.

Introduction

Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are minor metabolites of ethanol, measured most often in urine as relapse markers for alcohol ingestion (1–3). Studies regarding their analysis in whole blood, serum, and plasma have also been published (4–9). In these matrices, EtG and EtS are used to prove antemortem alcohol ingestion in postmortem cases (10,11) or to determine time of alcohol ingestion in, for instance, cases of drunk driving (4).

Analysis of a substance in whole blood will measure its total amount, including the amount within red and white blood cells. The vast majority of blood cells are erythrocytes, constituting ~40% of the total blood volume, whereas the white blood cells account for only ~1% (12). Analysis of a substance in serum will exclude the amount present in blood cells. As some substances are bound to or in erythrocytes and others do not enter these cells, concentrations of drugs will often differ between whole blood and serum (12,13). This distribution of a substance between the serum and erythrocyte fractions of the blood is important in forensic toxicology, where whole blood is the most commonly analyzed medium and the results are often compared with serum or plasma analyses.

The plasma/blood (P/B) or serum/blood (S/B) ratios are well known for many drugs, including ethanol (14–18). Because ethanol is distributed in the body water, its concentration is higher in serum, which contains 12–18% more water compared to whole blood (16). For EtG and EtS, there are no published data on these ratios. The aim of this study was therefore to determine the ratio between serum and blood for EtG and EtS by measurements in whole blood and serum from heavy drinkers prepared simultaneously.

Materials and Methods

Whole blood was collected, and serum was prepared from patients at admission to an alcohol rehabilitation clinic at Aker University Hospital (Oslo, Norway) as follows: the skin was first cleaned with isopropanol, and a needle was inserted into a cubital vein. Blood was collected in two separate Vacutainer tubes, one for blood and one for preparation of serum, using the same needle insertion. The time between the sampling of the two samples was therefore only a few seconds. The Vacutainer tube used for whole blood was identical to those used for routine samples at the Norwegian Institute of Public Health and contained 143 IU (0.286 mg) heparin and 20 mg fluoride. The Vacutainer tube was filled with 5 mL of blood, resulting in a very minor dilution factor (1:260). For the serum samples, the Vacutainer tube without additives was filled with 5 mL of blood.

For the serum samples, the Vacutainer tubes were centrifuged approximately 1 h after sampling, and 1 mL of serum was transferred to another container. All samples were stored...
EtG was analyzed in blood and serum using a previously published method (10). All validation details for EtG are given in this reference. EtS was analyzed in blood and serum using the same liquid chromatography–mass spectrometry (LC–MS) method as for EtG (10). EtS was supplied by TCI (Tokyo Chemical Industry Tokyo, Japan) and EtS-d5 by Lipomed (Cambridge, MA). The sample preparation included protein precipitation with cold methanol and followed by at least 10 min at −20°C, centrifugation, and evaporation of the organic phase to dryness. The residue was dissolved in water before injection on the LC-column Hypercarb separation column (2.1 × 100 mm, 5-µm particle size) and Hypercarb guard column (2.1 × 10 mm) from Thermo Electron (Cambridge, U.K.). The MS instrument, a Waters ZQ 2000 single-hexapole MS with an electrospray ionization (ESI) interface (Waters, Milford, MA), was operated in the negative mode for detection of EtS and EtS-d5. 

Results

From the present study, it seems that EtG and EtS are higher in serum than in whole blood, with median S/B ratios of 1.69 for EtG and 1.30 for EtS.

Discussion

This study showed that concentrations of EtG and EtS are higher in serum than in whole blood, with median S/B ratios of 1.69 for EtG and 1.30 for EtS. The ratio between serum and blood was somewhat higher than that of the mother substance ethanol, which was previously reported as 1.12 (14) and 1.14 (15). The ratio for ethanol is simply determined from the water content, which is lower in whole blood than in serum (14,16), as there is no evidence of ethanol binding to any components of blood (17). The EtG and EtS molecules share with ethanol the quality of water solubility, but are much larger molecules, and the situation is accordingly more complex.

From the present study, it seems that EtG and EtS are molecules with relatively little partitioning into red blood cells, and this could be caused by several qualities of the
molecules. For some drugs, like digoxin, lipophilicity is the most important factor determining the S/B ratio (20). As EtG and EtS are water soluble, this could be a reason why penetration into the red blood cells is difficult. Another reason could be the size of the molecules, as they are too big to enter through aqueous channels (12). The affinity for plasma proteins versus the affinity for binding sites inside the red blood cells is also important for the S/B ratio, and this ratio is therefore high for drugs like diazepam (21,22) and tetrahydrocannabinol (13,23). There are no reports on EtG or EtS binding to plasma proteins, indicating that this type of mechanism was not responsible for the skewed distribution of the ethanol metabolites.

The S/B ratio was significantly lower for EtS than for EtG, suggesting different properties of the two molecules. It is possible that EtS, given the smaller molecular size (M_w 126) compared to EtG (M_w 222), has better opportunity to enter erythrocytes.

Age, sex, and disease were previously reported not to influence distribution between erythrocytes and serum to a significant degree (12,24,25). None of the subjects in the present study showed serum/whole blood ratios that differed considerably from the rest of the group, and the variation was on the same order of magnitude as shown for ethanol (17).

The serum/plasma ratio for ethanol was previously reported as 1 (14). Unlike serum, plasma does not contain fibrinogen, but fibrinogen has not been reported to contain binding sites for any drugs (26–28). The amount of drugs present in plasma and serum is therefore expected to be almost identical, and this was also indicated for EtG and EtS (unpublished results).

To exclude analytical reasons for the different results obtained in blood and serum, calibration curves and controls at the same concentration levels were prepared from serum and whole blood reference samples, respectively, giving almost identical results for all samples. This was expected, considering the use of deuterated internal standards for both EtG and EtS in this method. The sampling of whole blood and serum from the patients was also made at the exact same time. Our findings are supported by a small number of data from previous kinetic studies of EtG in serum that show higher values than in blood under comparable conditions (4,7,29).

The difference in serum and whole blood concentrations of EtG and EtS could be of practical importance. Although there are both inter- and intradividual variations in EtG and EtS levels obtained from ingestion of a certain ethanol dose, a 70% higher result when measuring EtG in serum than whole blood would be important to realize. When comparing publications where similar ethanol levels lead to different EtG and EtS results, the serum/whole blood ratio should be remembered as one possible explanation. Also, if wider use of EtG and EtS evolves, a serum sample for clinical analysis and a whole blood sample for forensic analysis could be obtained from the same patient within the same period and analyzed for EtG and EtS, a situation that presently occurs for ethanol. It would then be relevant to have a simple explanation for different results.

In conclusion, the present study showed that the concentration of the ethanol metabolites EtG and EtS was higher in serum than in whole blood. This has to be considered when blood results obtained from forensic toxicology are compared to serum or plasma results from clinical laboratories.

References

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