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Evaluation of 1,5-anhydro-D-glucitol in clinical and forensic urine samples

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ABSTRACT

Because of the lack of characteristic morphological findings post mortem diagnosis of diabetes mellitus and identification of diabetic coma can be complicated. 1,5-Anhydroglucitol (1,5-AG), the 1-deoxy form of glucose, competes with glucose for renal reabsorption. Therefore low serum concentrations of 1,5-AG, reflect hyperglycemic excursions over the prior 1–2 weeks in diabetic patients. Next to clinical applications determination of 1,5-AG can also be used in forensic analysis. To investigate the elimination of 1,5-AG, a liquid chromatographic–mass spectrometric method for the determination of 1,5-AG and creatinine in urine was developed and validated according to international guidelines. To evaluate ante mortem concentrations of 1,5-AG spot urine samples of 30 healthy subjects, 46 type 1 and 46 type 2 diabetic patients were analyzed. 1,5-AG urine concentrations of diabetic patients were significantly ($p < 0.001$) lower (mean: 1.54 μg/ml, n = 92) compared to concentrations of healthy subjects (mean: 4.76 μg/ml, n = 30) which led to the idea that 1,5-AG urine concentrations post mortem might help in the interpretation of a diabetic coma post mortem. Urine of 47 deceased non-diabetics, 37 deceased diabetic and 9 cases of diabetic coma were measured. Comparison of blood and urine 1,5-AG concentrations in clinic samples (linear, $R^2 = 0.13$) and forensic samples (linear, $R^2 = 0.02$) showed no correlation. Urinary levels of 1,5-AG in deceased diabetic (mean 6.9 μg/ml) and in non-diabetic patients (mean 6.3 μg/ml) did not show a significant difference ($p = 0.752$). However, urinary 1,5-AG concentrations in deceased due to diabetic coma (mean: 1.7 μg/ml) were significantly lower than in non-diabetic (mean: 6.3 μg/ml, $p = 0.039$) and lower than in diabetic cases (mean: 4.7 μg/ml, $p = 0.058$). The determination of a reliable cut-off for the differentiation of diabetic to diabetic coma cases was not possible. Normalization of urinary 1,5-AG concentrations with the respective creatinine concentrations did not show any gain of information. In clinical (serum) and forensic blood samples a significant difference between all groups could be detected ($p < 0.05$). Comparison of blood and urine 1,5-AG concentrations in clinical samples (linear, $R^2 = 0.13$) and forensic samples (linear, $R^2 = 0.02$) showed no correlation.

1. Introduction

1,5-Anhydro-D-glucitol (1,5-AG) (Fig. 1), the 1-deoxy-form of glucose, is ingested by nutrition and competes with glucose for reabsorption by the sodium dependent glucose like transporter 4 (SGLT-4) in the kidneys [1]. Determination of 1,5-anhydroglucitol represents a complement to the determination of glycated hemoglobin ($HbA1_C$) as it more accurately predicts rapid changes in glycemia (postprandial hyperglycemia) than $HbA1_C$ or fructosamine [2].

In the living, the normal plasma 1,5-AG concentration can be lowered by inhibition of tubular reabsorption during periods of hyperglycemia. In normoglycemia 1,5-AG is maintained at constant steady state levels due to a large body reserve compared to the amount of intake by nutrition and due to lack of metabolism [1]. In hyperglycemic situations high blood glucose concentrations (especially when blood glucose exceeds the renal threshold of 160–180 mg/dl) inhibit the renal reabsorption of 1,5-Anhydroglucitol

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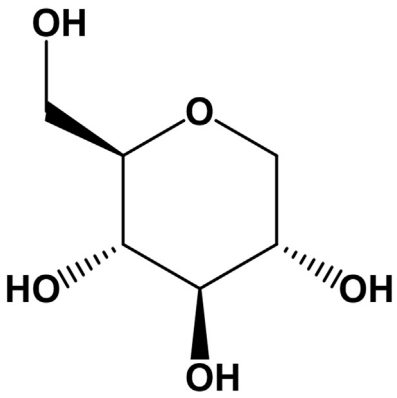

Fig. 1. Structure of 1,5-anhydro-D-glucitol (M = 164,2 g/mol).

from primary urine [1] and result in lower blood 1,5-AG concentrations [3]. Therefore patients with diabetes mellitus present significantly lower serum concentrations of 1,5-AG than non-diabetic patients [2–5]. 1,5-AG is metabolically stable and is not affected by age or body weight [6,7]. In Japan 1,5-AG is used as biomarker of glycemic control since 1990 [4,7,8]. Besides hyperglycemia, lower 1,5-AG levels are associated with prevalent retinopathy [9,10], cardiovascular disease and a higher mortality [11,12].

Post mortem diagnosis of diabetes and identification of diabetic coma can be difficult because of the lack of characteristic morphological findings and the fact that biochemical parameters are either difficult to interpret or just unusable [13,14]. Glucose might be an unreliable factor because it is rapidly metabolized into lactate by glycolysis after death. Therefore, post mortem biochemical evaluation of glucose metabolism should be based on glucose levels in vitreous humor (VH) [15,16]. Zilg et al. [17] postulated a threshold of >180 mg glucose per dL vitreous humor for the interpretation of a hyperglycemic death. Glycated hemoglobin (HbA1c) – although very stable after death [18] – is not a good indicator of glycemic control over shorter periods. Due to the irreversible formation and the stability HbA1c has a relatively long metabolic half-life and represents a good long time marker for glycemic control [19,20]. Due to the same reasons acutely changes in blood glucose levels cannot be observed in HbA1c values. In a previous study [21] it has been shown that post mortem 1,5-AG concentrations in femoral blood of diabetics were significantly lower than in non-diabetics. Deaths due to diabetic coma showed lower blood concentrations of 1,5-AG than diabetic deceased without metabolic derailment, however without statistical significance and concentrations were overlapping [21]. The total amount of urinary 1,5-AG in a glucose challenged rat was much higher than in the control rat [22] which led to the idea that the same significance could exist in the living and deceased and that a potential cut-off for 1,5-AG in urine post mortem for the determination of a diabetic coma could be established.

Analysis of 1,5-AG is mostly performed using the GlykoMark® assay (GlycoMark, Inc.) [1,2] or the Determiner-L-1,5-AG® assay manufactured by Kyowa Medex (Tokyo, Japan) [23], which produces comparable results [24]. In these two assays, a by-product is detected colorimetrically using a standard peroxidase assay after the oxidation of 1,5-AG to 1,5-anhydrofuctose by pyranose oxidase. Although these tests are sensitive, interference by glucose, bilirubin, hemoglobin [23], maltose [25] or myo-inositol [26] have been described. To bypass these interactions Tanabe et al. used a flow-injection system [27], in which glucose and other interfering substances were separated using an anion exchange column prior to the enzymatic detection. Gas chromatographic separation for determination of 1,5-AG has also been described [1]. The liquid chromatographic separation is often performed using polar stationary phases like amid, amino [28,29] or similar polar materials [30] combined with hydrophilic interaction liquid chromatographic (HILIC) eluent conditions. To improve the separation of disturbing substances high performance liquid chromatographs (HPLC), coupled with pulsed amperometric [5], fluorometric [31], enzymatic [32] or with mass spectrometric detection [21,28–30] are used. Onorato et al. showed that 1,5-AG forms acetate adducts and can be measured using MS^3 experiments. Due to its polar character 1,5-AG is weakly ionized by electro-spray-ionization (ESI) [28] but shows higher intensity using atmospheric-pressure-chemical-ionization (APCI) [21,29,30] in mass spectrometric analysis.

In order to investigate the renal elimination of 1,5-AG and the connection to urine concentrations some approaches of quantification in urine matrix are described [26,29,33]. Akanuma et al. found a significant correlation between glucose and 1,5-AG concentrations in urine [33]. Namba et al. collected serum and 24 h urine samples from 15 type 1 diabetics and found a linear correlation of urinary glucose concentration to the ratio of serum and urine 1,5-AG concentrations [26]. Onorato et al. investigated urine from 47 patients treated either with a placebo or with a proprietary compound known to alter levels of 1,5-AG and discovered an observable difference between these groups [29]. All of these approaches proved urinary 1,5-AG concentrations as valuable parameter by using 24 h urine to normalize the urinary 1,5-AG concentration by determination of the total amount per day.

The most accurate way to normalize urine concentrations is to collect 24 h urine, because spot urine samples only provide punctual insight in the excretion of the investigated substance [34]. An ideal normalization parameter for quantification in urine would include minimal intra- and interindividual variability and would not be influenced by external conditions. It is questionable whether creatinine fulfills all of these conditions [34,35]. However, due to the relative constant excretion [36], urinary creatinine concentrations are used to normalize the concentrations of several analytes in forensics.

In this study we investigated urinary 1,5-AG concentrations of living and deceased diabetic and non-diabetic persons. To our knowledge, the concentrations of 1,5-AG in urine after death have not been investigated yet and thus this biomarker could give the possibility to prove hyperglycemia that has been occurred prior to death.

2. Experimental

2.1. Analytical methods

2.1.1. Chemicals and reagents

1,5-Anhydro-D-glucitol (purity ≥ 98%)was purchased from Sigma (Steinheim, Germany). The internal standard (IS) 1,5-anhydro-D-[$^{13}C_6$] glucitol (purity ≥ 98%, 99 atom % ^{13}C) was purchased from Omicron Chemicals (South Bend, USA). Creatinine (purity ≥ 98%) and creatinine-D_3 (purity ≥ 98%, 99,9 atom % D) were purchased from LGC Standards (Wesel, Germany). Acetonitrile (from Merck, Darmstadt, Germany) and methanol (Sigma, Steinheim, Germany) were LC–MS grade. Water was purified using an ultrapure water system (Sartorius, Goettingen, Germany). All other substances were of analytical reagent grade.

2.1.2. Preparation of stock, standard and QC solutions

All solutions were prepared with ultrapure water. 1,5-AG and creatinine, stock solutions of 1 mg/ml and 40 mg/ml, respectively, were prepared. The IS stock solutions were prepared at concentrations of 5 mg/ml (creatinine-D_3) and 1 mg/ml (1,5-AG-$^{13}C_6$). The