

Chapter 2

Molybdenum Cofactor Deficiency: Metabolic Link Between Taurine and S-Sulfocysteine

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Abstract Molybdenum cofactor deficiency (MoCD) is a rare inherited metabolic disorder characterized by severe and progressive neurologic damage mainly caused by the loss of sulfite oxidase activity. Elevated urinary levels of sulfite, thiosulfate, and S-sulfocysteine (SSC) are hallmarks in the diagnosis of both MoCD and sulfite oxidase deficiency. Sulfite is generated throughout the catabolism of sulfur-containing amino acids cysteine and methionine. Accumulated sulfite reacts with cystine, thus leading to the formation of SSC, a glutamate analogue, which is assumed to cause *N*-methyl-D-aspartate receptor-mediated neurodegeneration in MoCD patients. Recently, we described a fast and sensitive HPLC method for diagnostic and treatment monitoring of MoCD patients based on SSC quantification. In this study, we extend the HPLC method to the analysis of hypotaurine and taurine in urine samples and no interference with other compounds was found. Besides the known elevation of SSC and taurine, also hypotaurine shows strong accumulation in MoCD patients, for which the molecular basis is not understood. SSC, hypotaurine, and taurine urinary excretion values from control individuals as well as MoCD patients are reported and over 20-fold increase in taurine urinary excretion was determined for MoCD patients demonstrating a direct link between sulfite toxicity and taurine biosynthesis in MoCD.

Abbreviations

Moco	Molybdenum cofactor
MoCD	Molybdenum cofactor deficiency
SOD	Sulfite oxidase deficiency

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SSC S-sulfocysteine
HPLC High-performance liquid chromatography

2.1 Introduction

Molybdenum cofactor deficiency (MoCD) is a rare inherited metabolic disorder (Johnson et al. 1980; Johnson and Duran 2001) caused by defects in the biosynthesis of the molybdenum cofactor (Moco) leading to the simultaneous loss of activities of all molybdenum-dependent enzymes: sulfite oxidase, xanthine dehydrogenase, aldehyde oxidase, and the mitochondrial amidoxime-reducing component (Schwarz et al. 2009). Affected patients exhibit severe neurological abnormalities, such as microcephaly and seizures, and they usually die in early childhood (Johnson and Duran 2001). Sulfite oxidase deficiency (SOD) is less frequent but clinically indistinguishable from MoCD, which renders sulfite oxidase as the most important Moco enzyme in humans (Tan et al. 2005). Sulfite oxidase catalyzes the oxidation of sulfite, which is generated throughout the catabolism of sulfur-containing amino acids, to sulfate (Griffith 1987; Johnson and Duran 2001). Deficiencies of Moco and sulfite oxidase result in the accumulation of sulfite, a highly toxic molecule that breaks disulfide bridges in proteins and cystine, thereby affecting many protein and cellular functions (Zhang et al. 2004). Sulfite accumulation is accompanied by the formation of secondary metabolites such as thiosulfate and S-sulfocysteine (SSC) (Johnson and Duran 2001), which together with reduced homocysteine levels (Sass et al. 2004) are common biochemical indicators for MoCD and SOD.

Sulfite is generated throughout the catabolism of sulfur-containing amino acids in two steps. First, the cytosolic enzyme cysteine dioxygenase catalyzes the formation of cysteine sulfinic acid (CSA). Second, either CSA undergoes a transamination in mitochondria, which leads to the formation of sulfite, or it is decarboxylated in the cytosol leading to the formation of hypotaurine, which is further oxidized to taurine. In MoCD sulfite first accumulates in liver, where most of the catabolism of sulfur-containing amino acids takes place. Subsequently, accumulation of sulfite in plasma is detectable and finally sulfite crosses the blood–brain barrier triggering a devastating and progressive neuronal damage (Schwarz et al. 2009).

Using a knockout animal model for MoCD (Lee et al. 2002) a substitution therapy with cyclic pyranopterin monophosphate has been established (Schwarz et al. 2004) and recently a first successful treatment for an MoCD (type A) patient has been reported (Veldman et al. 2010). Before treatment was initiated, a manifested rapid increase of urinary sulfite, thiosulfate, and SSC values was recorded. However, within few days after treatment was initiated, a remarkable normalization of all MoCD biomarkers as well as a significant clinical improvement of the patient were observed. Recently, we reported the development of a new HPLC method for diagnosis and treatment monitoring of MoCD patients, which enables an accurate and sensitive measurement of urinary as well as serum SSC levels and is being currently used to diagnose the disease to monitor treated patients (Belaidi et al. 2011).

2.2 Methods

2.2.1 Creatinine Analysis

Creatinine determination was based on the Jaffe method and carried out as previously described (Belaidi et al. 2011). Briefly, 50 μ l of diluted urine samples were mixed with 150 μ l alkaline picrate solution (1.2% picric acid in 0.75 M sodium hydroxide) and the formation of an orange-red complex between creatinine and alkaline picrate was quantified by measuring the absorbance at 490 nm.

2.2.2 HPLC

HPLC analyses were carried out on an Agilent 1200 SL system (Agilent Technologies GmbH, Boeblingen, Germany). The chromatographic conditions were identical to the previously reported SSC quantification method (Belaidi et al. 2011). Automated pre-column derivatization with O-phthalaldehyde (OPA) was used and the analyzed compounds were separated on a reversed-phase C18 column: XBridge (150 \times 4.6 mm, 3.5 μ m, Waters GmbH, Eschborn, Germany). For detection the UV absorbance at 338 nm was recorded and compound identification was achieved by comparing the retention time with that obtained for a standard. Peak area was used for calibration. SSC, hypotaurine, and taurine amounts were determined by standard addition and normalized to creatinine concentration.

2.3 Results

2.3.1 HPLC Determination of Hypotaurine and Taurine in Urine Samples

HPLC analysis of amino acids with OPA derivatization is one of the most sensitive methods for amino acid quantification with detection limits in the femtomole range. We previously developed a method for SSC determination in urine samples using pre-column derivatization with OPA, which resulted in fast and accurate measurement (Belaidi et al. 2011). In this study we extend the method to the measurement of hypotaurine and taurine in addition to SSC. Under the chromatographic conditions described above, separation was completed within 15 min using isocratic elution. Hypotaurine and taurine yielded sharp peaks eluting at 13.8 and 14.3 min, respectively, whereas SSC eluted at 8 min (Fig. 2.1a). Urine analysis in a sample derived from a healthy individual revealed the presence of very low amounts of SSC (4 mmol/mol creatinine), whereas hypotaurine and taurine levels were 25 and 30 mmol/mol creatinine, respectively (Fig. 2.1a). In contrast, analysis of a urine

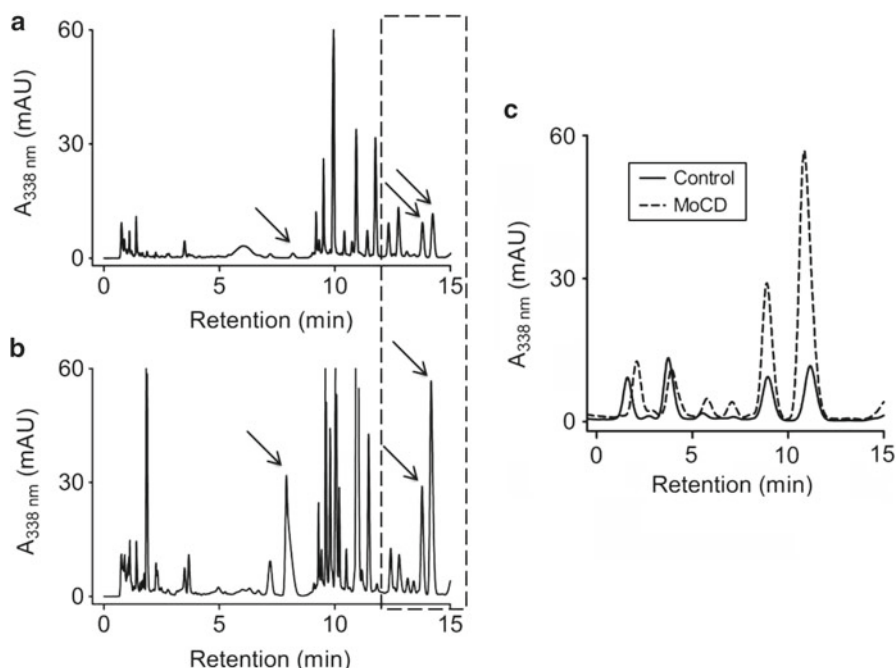


Fig. 2.1 HPLC analysis of hypotaurine, taurine, and SSC in a control individual (a) and an MoCD patient (b). An overlay of both chromatograms—control individual (solid line) and MoCD patient (dashed line)—highlighting hypotaurine and taurine peaks is shown in (c). SSC, hypotaurine, and taurine are highlighted in panels (a) and (b) by arrows

sample derived from an MoCD patient showed—in addition to an accumulated SSC peak—a clear accumulation of both hypotaurine and taurine (Fig. 2.1b). Comparison of the chromatograms derived from a healthy control sample (Fig. 2.1c, solid line) and an MoCD patient sample (Fig. 2.1c, dashed line) revealed a tenfold increase in the urinary excretion levels of hypotaurine and taurine in the MoCD patient (Fig. 2.1c).

2.3.2 Determination of Taurine Excretion Levels in Healthy and MoCD Patients

After confirming that hypotaurine and taurine excretion levels are up-regulated in an MoCD patient, the method was applied to the analysis of nine urine samples derived from MoCD patients as well as urine samples from control individuals. As expected, the SSC values were very low in control samples (1–9 mmol/mol creatinine), while hypotaurine and taurine levels ranged from 10 to 70 (median 21) and 30 to 100 (median 67) mmol/mol creatinine, respectively (Fig. 2.2a). In contrast, samples derived from MoCD patients showed, in addition to SSC accumulation

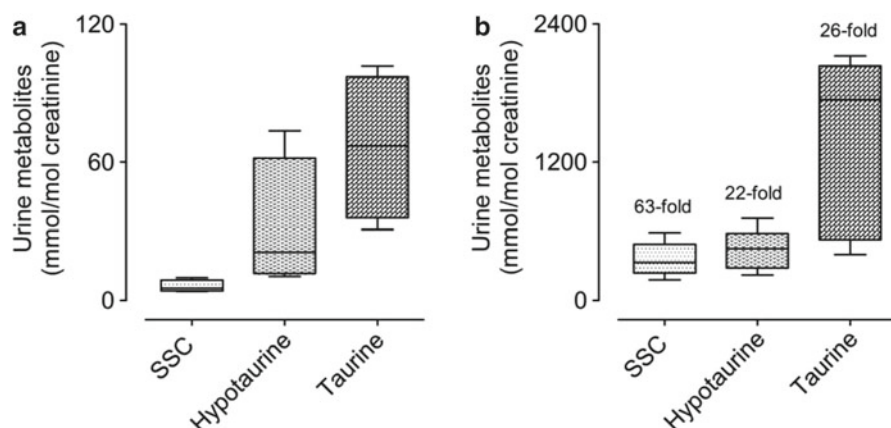


Fig. 2.2 Hypotaurine, taurine, and SSC urinary levels in control individuals and MoCD patients. Urine samples derived from control individuals and nine MoCD patients are shown in panel (a) and (b), respectively. In addition, numbers over the bars in panel (b) indicate the fold increase in the median value in MoCD patients for each metabolite. Metabolites in urine were normalized to creatinine concentration and horizontal bars show the median values

(180–600 mmol/mol creatinine, Fig. 2.2b), very high levels of both hypotaurine and taurine. The excretion levels of taurine were in the millimolar range and reached over 25-fold increase in the median value of healthy individuals, while a 21-fold increase in the median value of hypotaurine was found (Fig. 2.2b).

2.4 Discussion

MoCD is a rare metabolic disorder characterized by a severe and massive neurodegeneration leading to death in early childhood. SSC which is present at very low levels in healthy individuals (Johnson and Duran 2001; Belaidi et al. 2011) is one of the most elevated metabolites in MoCD patients and due to its structural similarity to glutamate, it is believed to act on NMDA receptors (Olney et al. 1975). In the past, many reports showed an important role of taurine in modulating glutamate and GABA signaling (El Idrissi and Trenkner 1999, 2004). Furthermore, taurine has been shown to prevent excitotoxicity through modulation of intracellular calcium homeostasis (El Idrissi and Trenkner 1999). Knowing the importance of calcium signaling in the glutamate-induced neurotoxicity and the fact that taurine and sulfite are both formed directly from CSA, we asked to which extent taurine is also affected in MoCD. We developed an HPLC method for the simultaneous detection of SSC, hypotaurine, and taurine in urine samples aiming to determine the excretion levels of those compounds in control and MoCD patients. Our results showed over 20-fold higher excretion values for hypotaurine and taurine in MoCD patients as compared

to control individuals. The fact that not only taurine but also hypotaurine, the direct precursor for taurine synthesis, are excreted in high levels in urine of MoCD patients provides evidence for an up-regulation of the entire taurine biosynthesis pathway from CSA via hypotaurine to taurine. Thus, an exclusive contribution of taurine transport is not the sole explanation. As a 64-fold increase in SSC levels was measured in MoCD patients, while only a 20-fold increase in both hypotaurine and taurine was found, we assume that sulfite-mediated SSC formation precedes the accumulation of taurine and hypotaurine, pointing to a more distal metabolic relationship. In summary, it remains unclear how sulfite and/or SSC contributes to this up-regulation. Due to the previously reported important role of taurine in preventing neurotoxicity (El Idrissi and Trenkner 1999), we speculate that taurine up-regulation may result from a compensatory effect to overcome the toxicity caused by SSC in the brain or a feedback inhibition of the sulfite branch in cysteine catabolism, thus leading to an increased taurine formation. Additional experiments are required to elucidate the effect of taurine, especially on the SSC-induced neurotoxicity.

2.5 Conclusion

Here we confirm the link between MoCD and taurine biosynthesis using a novel method for the simultaneous detection of SSC, taurine, and hypotaurine in healthy control individuals and MoCD patients. Interestingly, the analysis of urine samples derived from MoCD patients revealed over 20-fold increase in both hypotaurine and taurine levels as compared to control individuals, thus providing evidence for an up-regulation of the hypotaurine and taurine pathway and demonstrating a link between sulfite toxicity and taurine biosynthesis in MoCD patients. However, it remains unclear by which mechanisms taurine and hypotaurine are up-regulated in MoCD.

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Taurine 8

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