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Fatal hyperammonemia and carbamoyl phosphate synthetase 1 (CPS1) deficiency following high-dose chemotherapy and autologous hematopoietic stem cell transplantation

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ABSTRACT

Fatal hyperammonemia secondary to chemotherapy for hematological malignancies or following bone marrow transplantation has been described in few patients so far. In these, the pathogenesis of hyperammonemia remained unclear and was suggested to be multifactorial.

We observed severe hyperammonemia (maximum 475 μmol/L) in a 2-year-old male patient, who underwent high-dose chemotherapy with carboplatin, etoposide and melphalan, and autologous hematopoietic stem cell transplantation for a neuroblastoma stage IV. Despite intensive care treatment, hyperammonemia persisted and the patient died due to cerebral edema.

The biochemical profile with elevations of ammonia and glutamine (maximum 1757 μmol/L) suggested urea cycle dysfunction. In liver homogenates, enzymatic activity and protein expression of the urea cycle enzyme carbamoyl phosphate synthetase 1 (CPS1) were virtually absent. However, no mutation was found in *CPS1* cDNA from liver and *CPS1* mRNA expression was only slightly decreased. We therefore hypothesized that the acute onset of hyperammonemia was due to an acquired, chemotherapy-induced (posttranscriptional) CPS1 deficiency. This was further supported by *in vitro* experiments in HepG2 cells treated with carboplatin and etoposide showing a dose-dependent decrease in CPS1 protein expression. Due to severe hyperlactatemia, we analysed oxidative phosphorylation complexes in liver tissue and found reduced activities of complexes I and V, which suggested a more general mitochondrial dysfunction.

This study adds to the understanding of chemotherapy-induced hyperammonemia as drug-induced CPS1 deficiency is suggested. Moreover, we highlight the need for urgent diagnostic and therapeutic strategies addressing a possible secondary urea cycle failure in future patients with hyperammonemia during chemotherapy and stem cell transplantation.

1. Introduction

Hyperammonemia related to chemotherapy and hematopoietic stem cell transplantation (HSCT) is a known but rare and not well-understood condition and has so far been mainly described in hematological malignancies [1–5] (reviewed in [6]). In addition and not related to therapy but to the disease itself, multiple myeloma has been associated with an increased risk of hyperammonemia [7–9]. Besides the aforementioned conditions with an underlying malignant disease, hyperammonemia after unrelated cord blood transplantation was described in a 14-month-old boy with mucopolysaccharidosis type I [10].

The pathophysiology underlying the development of hyperammonemia in malignant disorders or during their treatment remained obscure in most cases. Following orthotopic lung transplantation and chemotherapy, hyperammonemia was suggested to be associated with reduced glutamine synthetase expression and activity [11,12]. Likewise, chemotherapeutic agents such as 5-fluorouracil [13,14] and L-asparaginase [4,15] were associated with the development of hyperammonemic encephalopathy resulting

Abbreviations: 3-OH-B, 3-Hydroxy-butyrate; AcAc, Acetoacetate; BW, Bodyweight; CA VA, Carbonic anhydrase VA; CPS1, Carbamoyl phosphate synthetase 1; HSCT, Hematopoietic stem cell transplantation; NAGS, *N*-acetyl-glutamate synthase; NCG, *N*-carbamyl-glutamate; OTC, Ornithine transcarbamylase; OXPHOS, Oxidative phosphorylation; PTM, Posttranslational modification; UCD, Urea cycle disorder.

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from yet not fully understood mechanisms. Generally, it was suggested that chemotherapy-induced hyperammonemia is caused by several factors including enhanced protein catabolism during fever and/or infection, parenteral nutrition with a high protein content, gastrointestinal bleeding and/or obstruction leading to increased intestinal ammonia production, urease-producing bacteria in the intestine or urinary tract and portocaval shunting [3,5,6].

We describe a 2-year-old boy who received high-dose chemotherapy with carboplatin, etoposide and melphalan and autologous HSCT to treat a neuroblastoma stage IV and who consecutively developed severe hyperammonemia with fatal cerebral edema. Since the patient's biochemical parameters suggested an impaired urea cycle function, we studied enzyme activities and protein expression and found deficiency of carbamoyl phosphate synthetase 1 (CPS1). Further investigations by *in vitro* experiments using HepG2 cells suggested that the CPS1 deficiency was secondary to the toxic effect of carboplatin and etoposide, hereby suggesting an explanation of the mechanism of chemotherapy-induced hyperammonemia.

2. Materials and methods

2.1. Patient

We report on a 2-year-old boy with a neuroblastoma stage IV, who died of fatal hyperammonemia following myeloablative chemotherapy with melphalan, etoposide and carboplatin given from day −7 according to protocol ANBL0532 (http://www.cancer.gov/clinicaltrials) (Fig. 1). The patient developed severe oral mucositis and gastrointestinal bleeding on day −3, requiring morphine, parenteral nutrition and transfusions of red blood cells and platelets. On day −2, he was in a reduced general health condition with high fever. Blood examination revealed severe aplasia. Antibiotic treatment with amikacin and ceftriaxone was initiated and replaced by meropenem and vancomycin on day 0 due to fever persistence; on the same day, stem cell transplantation was performed without complications. On day −1, the patient developed renal failure with a marked increase in serum creatinine levels and urinary loss proteins and glucose. Acute tubulo-interstitial nephritis was diagnosed that was thought to be secondary to several potentially nephrotoxic drugs (e.g. amikacin, chemotherapeutics). Acid–base status revealed low values for pH, pCO2 and bicarbonate but a normal anion gap, thus reflecting renal and/or intestinal bicarbonate loss. To avoid its potential nephrotoxicity, vancomycin was replaced by linezolid on day +3. Two days later, the patient suffered from a first focal seizure which prompted cranial computer tomography that was normal. An evaluation of venous blood samples revealed normal blood gas analysis, liver transaminases, bilirubin values and a prothrombin time in the upper normal range but persistent pancytopenia. On day +7, the patient suffered again from seizures and rapidly developed deep coma. Blood ammonia levels showed a peak value of 475 μmol/L (ref. <50) on day +8 concomitant with hyperlactatemia (maximal lactate 9.2 mmol/L on day +9; ref. 0.8–1.8). Plasma amino acid analysis revealed a highly elevated glutamine level (1757 μmol/l; ref. 250–820), whereas citrulline and arginine were in the normal range. Orotic acid in urine was absent on several occasions. Therapy with the nitrogen scavenger sodium-benzoate (250 mg/kg bodyweight (bw)/day) as well as L-arginine (2 mmol/kg bw/day) was started (day +8). When ammonia levels remained elevated, hemodialysis was initiated on the same day. At that time, electroencephalogram was already altered showing flat activity. A trial with *N*-carbamyl-glutamate (NCG) (200 mg/kg bw/day) was given on day +9 and stopped after 2 days due to persistent hyperammonemia. Brain magnetic resonance imaging on day +15 showed severe cerebral edema compatible with hyperammonemic encephalopathy. A diagnostic liver biopsy was performed on day +16, 3 days prior to death of the patient, revealing no further signs of liver disease.

2.2. Urea cycle enzyme assays

N-acetyl-glutamate synthase (NAGS), CPS1 and ornithine transcarbamylase (OTC) enzyme activities were determined in two different liver biopsy samples. One sample was obtained on day +16, the other sample postmortem. Tissue was immediately frozen at −80 °C, and enzyme assays were performed as described previously [16,17]

2.3. Western blot

Western blot of cell lysates obtained from cell culture experiments in HepG2 cells or from manually homogenized liver tissue in NP40 lysis buffer (Roche Diagnostics GmbH, Mannheim, Germany) was performed in an adapted procedure as described previously [18]. Briefly, protein concentrations were determined by Bradford assay [19]. Thirty micrograms of protein per lane was loaded on a 10% SDS polyacrylamide gel. The separated proteins were transferred on a nitrocellulose membrane, which was washed with TBS Tween 0.1% buffer and blocked in 5% milk. Primary antibodies (goat polyclonal anti-CPS1 antibody, sc-10516, and mouse monoclonal anti-GAPDH IgG, sc-47724, 1:50 and 1:500 in milk, respectively, both Santa Cruz Biotechnology, Heidelberg,

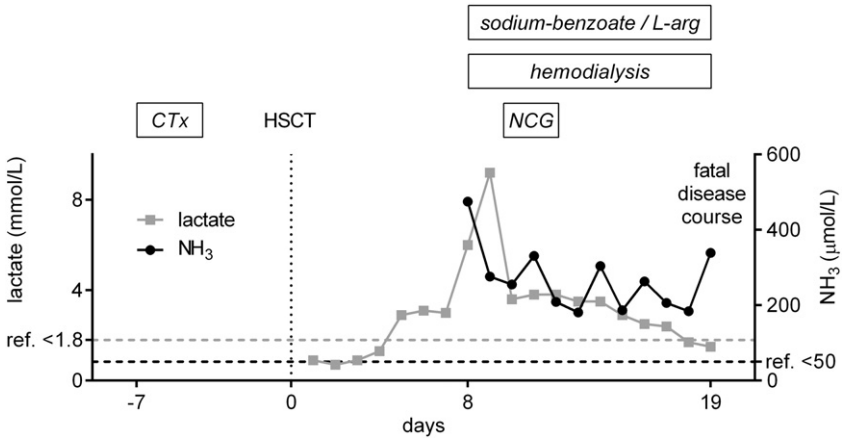

Fig. 1. Time course of main events during myeloablative chemotherapy. For further details, see text. On day −7, myeloablative chemotherapy (*CTx*) according to the protocol ANBL0532 (conditioning with melphalan from day −7 to −5, etoposide and carboplatin from day −7 to −4) was initiated followed by autologous hematopoietic stem cell transplantation (HSCT) on day 0. On day +8, hyperammonemia (and prominent hyperlactatemia) was diagnosed and treated with sodium-benzoate and L-arginine-hydrochloride (L-arg) for a duration indicated by boxes. Due to persistent hyperammonemia, hemodialysis was initiated also on day +8; from end of day +9, a trial with *N*-carbamyl-glutamate (NCG) was started and stopped after 48 h due to persistent hyperammonemia. Representative ammonia (black line with dots) and lactate (grey line with small boxes) levels from the patient are shown as well as the upper limits of normal for ammonia (dotted black line) and lactate (dotted grey line).