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Fatal liver failure after therapeutic doses of paracetamol in a patient with Duchenne muscular dystrophy and atypical pharmacogenetic profile of drug-metabolizing enzymes

Running title: Fatal liver failure therapeutic paracetamol

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Abstract

Paracetamol has a good safety profile, but pharmacogenetic differences in drug-metabolizing enzymes may have an impact on its risk of hepatotoxicity. We present a case of fatal acute liver failure (ALF) after therapeutic doses of paracetamol in a patient with Duchenne muscular dystrophy, where pharmacogenetic screening was conducted.

This 30-year-old man was electively admitted for a tracheostomy. A total of 14.5 g paracetamol was given over four days. He developed a severe ALF and died 11 days after admission. Pharmacogenetic screening showed absent CYP2D6 metabolism and increased CYP1A2 activity, which may have increased the formation of toxic intermediate metabolite, N-acetyl-p-benzo-quinone imine (NAPQI). He also had decreased function of UGT2B15, which increases the amount of paracetamol available for metabolism to NAPQI. Having a reduced muscle mass and thus a reduced glutathione levels to detoxify produced NAPQI may add to the risk of toxicity.

This case may indicate that pharmacogenetic variability is of potential relevance for the risk of paracetamol-induced hepatotoxicity in patients with neuromuscular diseases. Further studies should investigate if pharmacogenetic screening could be a tool to detect potentially increased risk of hepatotoxicity in these patients at therapeutic doses of paracetamol, and hence provide information for selection of analgesic treatment.

Introduction

Neuromuscular diseases (NMD) are a group of diagnoses that includes a wide range of disorders of the central nervous system (anterior horn cells or axon), the neuromuscular junction and/or muscle, leading to impaired muscle function [1]. Although each individual disorder in this group is rare, approximately 1:1000 individuals worldwide are affected by some type of NMD [2]. A number of recent studies indicate that pain is a significant problem in many NMDs [3-7] . Few studies have focused on the safety and efficacy profiles of analgesics for this group. Clinical experience indicates extensive use of over-the-counter medication such as paracetamol and ibuprofen in patients with NMD.

Paracetamol is a drug with a good safety profile in normal doses, which can be used for both acute and chronic pain. However, individual case reports of a hepatotoxic effect of paracetamol given in therapeutic doses to patients with NMDs have been published [8-11]. It remains unclear why some patients develop acute liver failure (ALF) on therapeutic doses of paracetamol. Pharmacogenetic variability may play a role for the vulnerability of ALF [12], but pharmacogenetic screening was not performed in any of the previously published cases. Paracetamol is subjected to metabolism via several phase I and phase II enzymes exhibiting genetic polymorphism, including CYP1A2, CYP2D6 and UGT2B15 [13-15]. We present a patient with NMD who developed fatal acute liver failure (ALF) following the administration of therapeutic doses of paracetamol. In this case, pharmacogenetic analyses of drug-metabolizing enzymes were conducted in order to search for possible explanations for this unexpected fatal outcome.

Case description

A 30-year-old man (50 kg) with Duchenne muscular dystrophy (DMD) was electively admitted for a tracheostomy. He suffered from cardiomyopathy due to his DMD and was treated with ramipril and bisoprolol. He also suffered from chronic respiratory failure type II, and used a non-invasive mechanical ventilator at home. In addition, he used lactulose on a regular basis for constipation.

On the day of admission (day 0), ALAT was slightly elevated with 101 U/L (reference range 10-70 U/L) and he had a low creatinine level of 7 µmol/L (range 60-105 µmol/L), consistent with his low muscle mass (Table 1). The tracheostomy procedure was conducted without any complications on day one, and paracetamol was initiated as analgesic therapy. Over the next four days, he was given a total dose of 14.5 g paracetamol (Figure 1). On the first day, he was given 3 g paracetamol intravenously (IV), on day two 4 g orally (PO), on day three 2 g IV and 2 g PO, on day four 3 g PO and on the morning of day five one dose of

0.5 g PO. The doses were given as 10 doses of 1 g paracetamol and 9 doses of 0.5 g in combination with 30 mg codeine. On the evening of day 4, he became hypotensive and had reduced urine production. He was given fluid and furosemide. On day 5, he developed gastrointestinal features with nausea and vomit. His urine was dark, diuresis low and he was transferred to the ICU for better monitoring with a tentative diagnosis of subileus. Routine blood samples revealed severe liver failure with ALAT 8921 U/L (10-70 U/L), INR 4.3 (<1.3), LD 5754 U/L (105-205 U/L), bilirubin 48 µmol/L (5-25 µmol/L) and ammonium 207 µmol/L (11-32 µmol/L) (Table 1). Serum paracetamol was elevated, peaking at 282 µmol/L (33-133 µmol/L) on day 5. A standard intravenous N-acetylcysteine regimen was therefore initiated (150 mg/kg over 1 hour, 50 mg/kg over 4 hours, 100 mg/kg over 16 hours). During the next days, he became unconscious and mechanical ventilation was initiated. He was rejected for liver transplantation due to the limited life expectancy related to his DMD. He developed circulatory failure in need of vasopressor and increasing respiratory failure. On day 11, all active treatment was withdrawn, and the patient died on the same day.

He was not given any other hepatotoxic medications and no drug interaction could explain his ALF. The cause for his ALF thus remained unclear and pharmacogenetic analyses were therefore retrospectively performed.

Methods

The following genes were analysed by the next-generation sequencing (NGS) system Ion Torrent Ion S5 and the AmpliSeq Pharmacogenomics Research Panel (Thermo-Fisher Scientific Waltham, Massachusetts, USA): UGT2B15 (encoding a glucuronidation enzyme), SLCO1B1 (encoding a hepatic uptake transporter), CYP3A5/3A4/2C19/2C9/2C8/1A2/2B6/2D6 (encoding respective oxidation enzymes). The number of single nucleotide polymorphism (SNPs) analysed of the respective genes were 1, 2, 10, 10, 11, 14, 2, 15, 8 and 21. This comprehensive pharmacogenetic analysis showed that the patient was a homozygous carrier of a SNP (rs3892097) encoding absent CYP2D6 metabolism (poor metabolizer) [16], and homozygous carrier of a SNP (rs762551) encoding increased CYP1A2 activity [17]. Further, the patient was a heterozygous carrier of SNPs in SLCO1B1 (rs2306283) and UGT2B15 (rs1902023), encoding partly increased and reduced activities, respectively [18, 19]. The patient was a heterozygous carrier of a variant in UGT2B15 gene (rs1902023), probably encoding reduced glucuronidation activity of paracetamol. SLCO1B1 genotype determining the phenotype of the OATP1B1 hepatic uptake transporter is probably not relevant for paracetamol pharmacokinetics. For the other analysed genes, no variant SNPs were detected in the NGS panel.

Discussion

This case report describes a fatal ALF in a patient with DMD given therapeutic doses of paracetamol over four days. Pharmacogenetic analysis showed poor metabolism of CYP2D6, increased CYP1A2 activity and decreased function of UGT2B15, all, which may alter the metabolic profile of paracetamol.

The recommended dose of oral paracetamol is usually maximum 4 g daily for up to two weeks, thereafter 3 g daily [20]. The oral bioavailability of paracetamol is 60-100% [20] and a lower maximum intravenous dose is therefore recommended. For patients weighing 50 kg or below, maximum recommended IV dosage is 15 mg/kg four times daily [20]. This patient weighed 50 kg and the maximum dose of IV paracetamol should be 3 g, as was given on day 1 and 2 g IV on day 3 (Figure 1). The recommended paracetamol dosage based on body weight was therefore not exceeded.

Paracetamol is metabolized in a phase I reaction to N-acetyl-p-benzo-quinone imine (NAPQI), which is causing hepatotoxicity (Figure 2). This reaction is catalyzed by the cytochrome P450 enzyme system. The CYP isoforms CYP1A2, CYP2A6, CYP2D6, CYP2E1 and CYP3A4 are all capable of catalyzing this reaction [12], but the relative importance of these enzymes in the formation of NAPQI is unclear [21]. The fact that the patient expressed altered metabolic activity of both CYP2D6 (abolished) and CYP1A2 (increased) may be of interest in this case, as CYP1A2 exhibits a higher catalytic activity towards paracetamol oxidation than CYP2D6 [22]. Therefore, the absent CYP2D6 metabolism increased the availability of paracetamol for CYP1A2-mediated formation of NAPQI. A possible higher formation of this toxic metabolite may have increased the patient's susceptibility of paracetamol-induced hepatotoxicity. Furthermore, the encoded decreased function of UGT2B15 [23], which is the major enzyme responsible for paracetamol metabolism by catalyzing the formation of an inactive glucuronide metabolite, may have increased substrate availability for the formation NAPQI [24]. Measurement of paracetamol metabolites could have confirmed this, but these analytical methods were not available.

Overall, the pharmacogenetic profile of our patient may potentially have played a role for the triggered ALF with therapeutic doses of paracetamol. It is therefore clinically relevant to characterize the pharmacogenetic profiles of patients subjected to paracetamol-induced ALF within therapeutic doses to investigate if there are any common traits. CYP2E1 is proposed to be the most important enzymes in the formation of NAPQI. However, the knowledge on the pharmacogenetic variability of the enzyme in relation to metabolic activity is limited [25]. Thus, it was not included in the NGS panel applied when characterizing the patient's pharmacogenetic profile. This was also the case for the

glutathione-*S*-transferases (GSTs) involved in the detoxification of NAPQI. However, it will be of interest to include these genes as well in pharmacogenetic studies investing multiple cases of toxicity with therapeutic dosing of paracetamol.

Seven cases of paracetamol toxicity in patients with NMD have been described in four previous reports [8-11], all related to postoperative pain medication or critical illness. Hynson (1999) described a 12-year-old male with Duchenne muscular dystrophy (DMD) who underwent spinal fusion and tendon-release surgery [8]. Pearce (2008) reported a 20-year-old male with DMD and epilepsy and a 42-year-old male with Limb-Girdle muscular dystrophy (LGMD), both critically ill with respiratory distress [9]. Ceelie (2011) reported three cases [10]. Two of them were
12-year-old patients with Spinal muscular atrophy (SMA) type II, a boy and a girl. They both underwent spinal fusion surgery for correction of scoliosis. The third patient was a 17-year-old girl with congenital muscular dystrophy (CMD) and carnitine deficiency admitted to the ICU with pneumonia and respiratory insufficiency [10]. Brehm (2019) reported a 26-year-old male with SMA type III who developed neutropenic fever three weeks after a femur fracture [11]. All seven patients reported above developed ALF following standard recommended doses of paracetamol. Only the girl with CMD reported by Ceelie died [10]. Pharmacogenetic screening was not done in any of these cases. All four reports advise physicians to be alert when prescribing paracetamol to patients with underlying myopathies.

Our case is the eighth case reported of ALF following therapeutic doses of paracetamol given to patients with NMDs. This is a patient group with low muscular mass and at risk of reduced levels of glutathione [26]. With such lower levels, less NAPQI will be converted to non-toxic metabolites. In theory, this could be a contributing factor for the development of ALF in these cases. Given the number of patients with NMD worldwide being treated with paracetamol and limited number of ALF cases, this is unlikely to be the only explanation. However, without a pharmacogenetic profile for the other seven patients, it is difficult to rule out the lower levels of glutathione as a contributing factor. Compared with the other postoperative patients, our patient was the only one who died. He had a pharmacogenetic profile with increased production of NAPQI, increased paracetamol available for metabolism to NAPQI and by adding a possible low level of glutathione; this may explain why he developed fatal ALF.

Based on the case reports in the literature and our patient, pharmacogenetic screening of this patient group may be a simple and cheap step to rule out patients who should avoid paracetamol.

Conclusion

We present a patient who was a poor metabolizer of the enzyme CYP2D6 and rapid metabolizer of CYP1A2. In addition, he had decreased function of UGT2B15. This, together with the NMD and likely low level of glutathione, may explain his fatal acute liver failure. Further studies should investigate if pharmacogenetic screening could be a tool to detect potentially increased risk of hepatotoxicity in patients with neuromuscular diseases during use of paracetamol in therapeutic doses, and hence provide information for selection of analgesic treatment in these patients.

Ethics

The closest relatives have consented to the publication of the case report.

Conflict of interest

The authors declare no conflict of interest.

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Table 1: Changes in laboratory results

Day	0	1	2	3	4	5	6 morning	6 evening	7
INR	1	-	1.2	-	-	4.3	6.5	3.2**	3
ALAT (U/L)	101	-	83	86	-	8921	7437	6547	6258
ASAT (U/L)	-	-	68	-	-	10987	8640	5727	4578
Bilirubin (µmol/L)	-	-	9	5	-	48	44	43	43
GT (U/L)	-	-	143	-	-	190	179	169	164
LD (U/L)	-	-	176	-	-	5754	3997	-	1174
Ammonium (µmol/L)	-	-	-	-	-	207	335	181	154
Creatinine (µmol/L)	7	-	< 5	< 5	-	14	9	-	8
Urea (mmol/L)	-	-	2.6	1.8	-	3.7	2.8	-	1.3
Paracetamol (µmol/L)	-	-	221*	156*	-	282	217	-	28

* retrospectively analyzed from original samples

** plasma given before analysis