Global metabolomics reveals severe 3-nitropropionic acid intoxication in a Norwegian patient

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INTRODUCTION

The neurotoxin 3-nitropropionic acid (3-NPA) is produced by fungi that can infest plants and vegetables (1). Succinate dehydrogenase, a key enzyme both in the citric acid cycle and the mitochondrial respiratory chain, is inhibited by 3-NPA (2). This inhibition can lead to symptoms in humans with varying severity, ranging from stomachache and headache to dystonia, coma and death. There is no known antidote for 3-NPA intoxication.

Human 3-NPA intoxications have mostly been reported from China. Among 884 cases, 88 fatalities were reported in China between 1972 and 1989 (1). Ingestion of moldy sugarcanes infected with *Arthrinium* spp., which can produce 3-NPA in toxic amounts, is the main identified source. In western parts of the world, there have been few published 3-NPA cases. Our report is the first of 3-NPA in Norway. We here present our metabolomics analysis both diagnosing a gravely ill 14-year-old Norwegian boy to suffer from acute 3-NPA intoxication, and providing a broad metabolic mapping of the widespread effects of the intoxication in our patient.

METHODS

Heparin plasma, urine and cerebrospinal fluid samples from various phases of the intoxication and reconvalescence were analyzed using LC-MS. Peak areas of metabolites in samples from the various phases were compared. Additionally, we analyzed a control sample from a healthy volunteer and an analytical reference standard of 3-NPA. All metabolomics analyses were performed with our global metabolomics LC-MS platform, with reversed phase chromatography and a Q Exactive Orbitrap mass spectrometer (3).

RESULTS

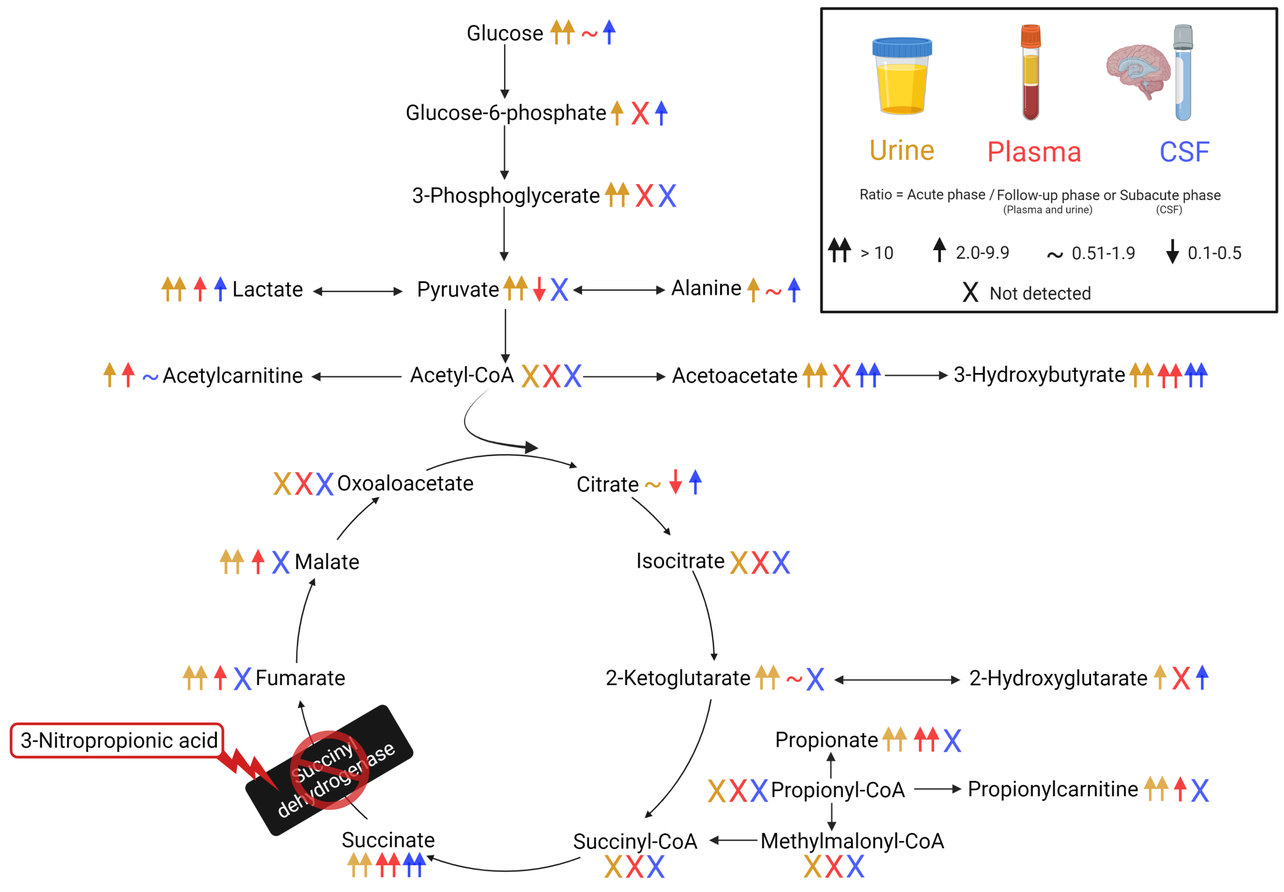

FIGURE 1 Simplified representations of the glycolysis pathway and citric acid cycle, showing altered metabolites in plasma, urine and cerebrospinal fluid (CSF) samples. The subacute phase CSF sample was taken 2 days later than the acute phase. The follow-up samples were taken 9 and 12 months later than the acute phase samples for plasma, and 12 months later for urine samples.

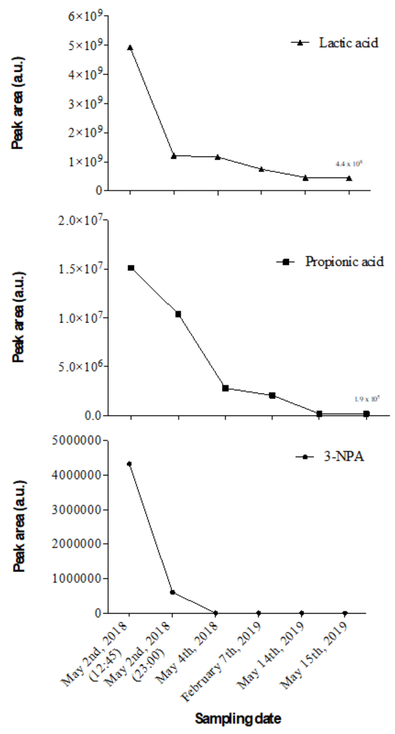

FIGURE 2 Peak area of lactic acid, propionic acid and 3-NPA in the patient plasma samples. The figure shows how the level of 3-NPA decreased from the first to the second sample 10 hours later during the acute phase (day 2), and that 3-NPA was not detected in samples obtained the morning of day 4 or thereafter.

3-NPA was detected and verified using an analytical reference standard in samples from the acute phase when the patient was severely ill. There was no detection of 3-NPA, nor a compound with the same *m/z*, fragmentation pattern and retention time, in the control sample. Annotated or identified metabolites with a ratio ≤0.50 or ≥2.0 between samples from two different clinical phases were reported in our publication (4). We identified large fluctuations in the amounts of many metabolites in the patient samples (80, 160, and 62 in plasma, urine, and cerebrospinal fluid samples, respectively), many of which belong to the citric acid cycle and closely associated biochemical pathways.

Figure 1 shows a simplified depiction of the glycolysis and citric acid cycle with altered metabolites highlighted. Several metabolites showed the same decreasing peak area pattern as 3-NPA in the patient samples, exemplified with lactic acid and propionic acid in plasma in **Figure 2**. As shown, the amount of 3-NPA rapidly decreased from the first to the second sample taken 10 hours later. In the following samples, 3-NPA was not detected, indicating rapid degradation and elimination of the toxin. A full forensic toxicology screening panel test performed on samples taken during the acute phase was performed prior to our analyses, with no detection of any toxicants. Despite a thorough investigation, the source of 3-NPA is still unknown in our case.

CONCLUSIONS

The present report illustrates the power of global metabolomics, as no toxicants were detected in the forensic toxicology screening of samples from our patient while our global approach revealed the source of illness. A global approach like ours enables unexpected findings, as it does not include a pre-defined, and thus limited, list of analytes. The present report (4) is the first on 3-NPA in Norway, and the only metabolomics study on human 3-NPA intoxication worldwide. As 3-NPA has caused intoxications in both Denmark (5) and Norway in recent years, inclusion of 3-NPA in forensic toxicology screenings in Europe and probably worldwide should be considered.

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