Int J Legal Med (2000) 113:171–174

CASE REPORT

S. Iwersen-Bergmann · A. Schmoldt

Acute intoxication with aniline: detection of acetaminophen as aniline metabolite

Received: 1 March 1999 / Received in revised form: 19 May 1999

Abstract A 47-year-old woman unwittingly ingested an unknown substance together with her breakfast coffee. She suffered effects such as strong headache, generalized cyanosis, and a burning sensation of the lips and collapsed some minutes later. After admission into hospital a methemoglobin level of 35% was determined in the blood. Treatment by administration of tolonium chloride (toluidine blue) resulted in complete recovery of the patient. The toxic agent was identified as aniline by GC with mass selective detection after organic solvent extraction and 11 h after ingestion the plasma aniline level was 0.13 mg/l. Acetanilide (0.79 mg/ml) and acetaminophen (2.3 mg/ml) were identified in plasma as metabolites of aniline. It was assumed that a high metabolic capacity for acetylation protected the victim from more severe reactions. Her husband confessed later that he had tried to poison her.

Key words Aniline · Poisoning · Plasma level · Metabolism

Introduction

Aniline and aniline derivatives are used in the chemical industry throughout the world mainly as intermediary products and solvents in the dyestuff industry. Three types of consumer products (indelible ink, shoe dye and wax crayons) have produced many cases of intoxication but poisoning by pure liquid aniline is almost unknown. The lethal oral dose is thought to be between 6–30 ml of pure aniline [4], and death is due to cardiovascular collapse. Aniline, like other nitro and amino derivatives of aromatic hydrocarbons, induces methemoglobinemia. The correlation between the severity of the intoxication and the methemoglobin level is only moderately good [5]. Since vomiting frequently occurs early after ingestion, it is difficult to estimate fatal doses and up to now no information about plasma concentrations of aniline was available. Here we report the course of an aniline intoxication and for the first time the corresponding plasma levels of aniline and its metabolites. Additionally acetaminophen was identified for the first time to be an aniline metabolite in humans.

S. Iwersen-Bergmann (✉) · A. Schmoldt
Department of Legal Medicine, University of Hamburg,
Butenfeld 34, D-22529 Hamburg, Germany

Materials and methods

Urine and blood samples were screened for basic drugs and drugs of abuse by immunoassay using the Boehringer Mannheim CEDIA with Hitachi 911 analyzer and Abbott ADx systems according to the manufacturers instructions. General unknown screening for drugs was performed after alkaline or acid liquid-liquid or solid-phase extraction (SPE) using thin-layer chromatography (TLC) [18], GC, gas chromatography-mass spectrometry (GC-MS) [14] and high-performance liquid chromatography (HPLC) [2]. Methemoglobin was determined according to Evelyn and Malloy [3].

For the determination of aniline and its metabolites, blood and urine samples were diluted with one part of water. Urine was analyzed untreated and after incubation with glucuronidase/arylsulfatase (1 h at 60 °C) for the cleavage of mainly acetaminophen glucuronides. After dilution, the samples were ultrasonicated for 30 min, then centrifuged. The supernatant was first extracted at pH 7 and again at pH 9 with ethyl acetate. The organic phases were combined and dried at 40 °C under a slow stream of nitrogen. The sample was reconstituted with 50 µl ethyl acetate and 1 µl was injected (splitless mode) into a Hewlett Packard (HP) gas chromatograph 5890 coupled to a HP 5972 mass selective detector. Helium was used as carrier gas with a flow rate of 1 ml/min. A HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm) was used. The operating temperatures for injector and detector were 200 °C and 280 °C, respectively. The oven temperature program was 50 °C for 3 min, 10 °C/min to 280 °C and the final temperature was maintained for 12 min.

Calibration curves for aniline, acetanilide and acetaminophen were determined with 1 mg/l p-nitrophenol as the internal standard. The ratios of the peak areas of aniline, acetanilide, and acetaminophen to p-nitrophenol were utilized to calculate the concentration of these analytes in specimens. Standard calibration curves were linear over the range of 0.1–100 mg/l.

The limit of quantitation was about 0.05 mg/l for aniline and acetaminophen and 0.03 mg/l for acetanilide. The correlation coefficient for each standard curve was 0.998 or greater.

To assure that no p-nitrophenol was in the sample possibly resulting from aniline metabolism, specimens were analyzed without internal standard before use.

Case report

A 47-year-old woman suffered from a burning and numb sensation of the lips after drinking her breakfast coffee and threw the rest of the coffee into the sink. A short time later she complained of strong headache, pulsing noises in the ears, lips still feeling numb and burned. Prior to admission into hospital she collapsed twice. The day before she had recognized a bitter soapy taste of the breakfast coffee and her daughter had noticed that the lips had turned blue. Furthermore she had developed a headache but no other symptoms had occurred.

On arrival at the hospital she was orientated and responsive but complained of strong headache. She showed cyanosis that was most pronounced at the lips, tongue and mucous membranes but the pulse rate and blood pressure were normal. After exclusion of cardiac or pulmonary diseases as a reason for the cyanotic state the determination of methemoglobin showed a level of 35% and Heinz bodies were found in 2 out of 1000 erythrocytes. The extent of oxidative hemoglobin denaturation is shown by Heinz body formation. The Heinz body counts were low but healthy humans do not show the presence of any Heinz bodies at all. The woman lived separated from her husband who was a chemical worker. Therefore an attempted poisoning with a methemoglobin forming agent was suspected.

Toxicological examinations which were carried out immediately showed the presence of aniline on the side of the coffee cup and in the water in the sink.

Treatment with 300 mg tolonium chloride and 1000 mg ascorbic acid led to an improvement in the cyanosis and a fall in the methemoglobin level. The next day the methemoglobin level was only 3% and Heinz bodies were measured at 3/1000 erythrocytes. When discharged 3 days later the patient had nearly fully recovered.

The plasma aniline level 11 h after ingestion was 0.13 mg/l and the metabolites acetanilide (0.79 mg/l) and acetaminophen (2.3 mg/l) were detected. The concentrations detected in urine were 3.2 mg/l aniline, 4.3 mg/l acetanilide, 106 mg/l acetaminophen and 315 mg/l acetaminophen conjugates. The methemoglobin-generating aniline metabolite phenylhydroxylamine (PHA) is susceptible to oxidation, rearrangement and disproportionation. It was not possible to detect PHA with our method, probably because it was isomerized to the corresponding aminophenol. P-aminophenol was detected in traces, o-aminophenol was not detectable (Fig. 1).

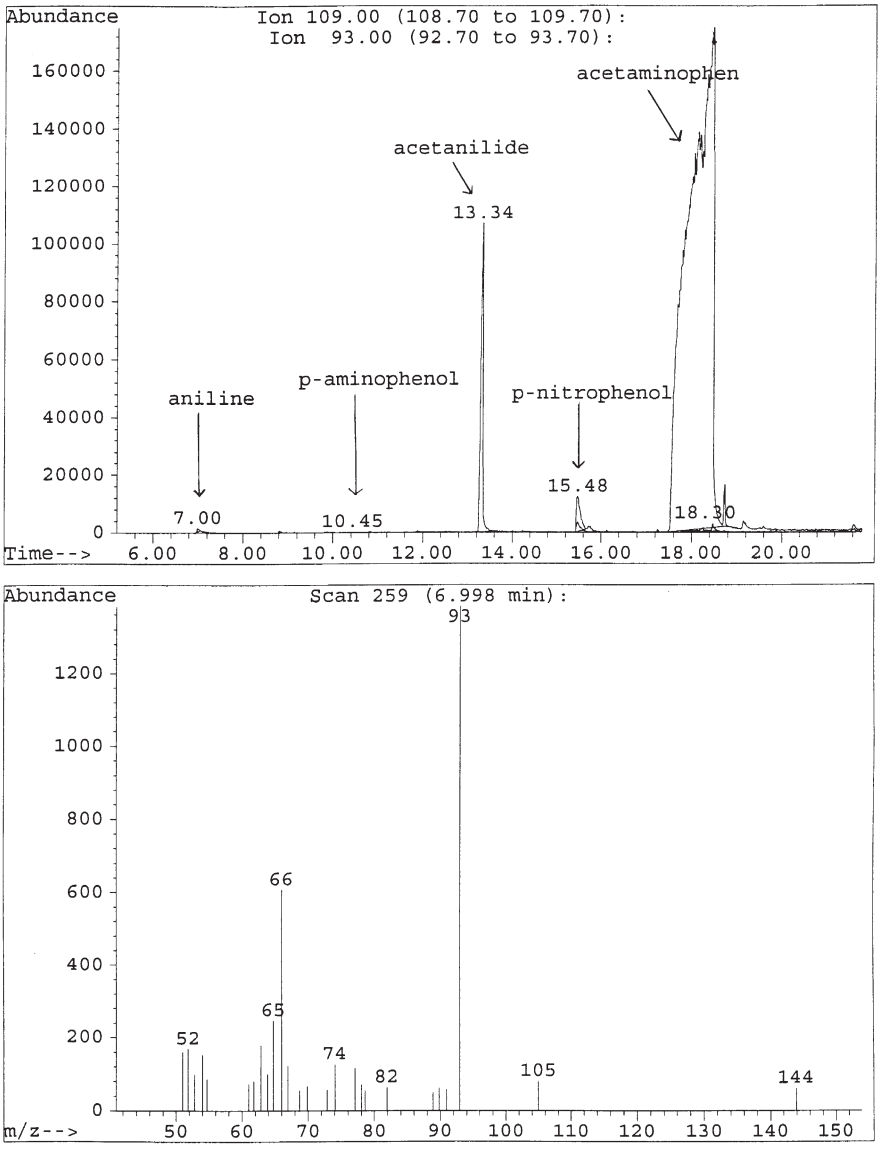

Fig. 1 Chromatogram recorded by the analysis of the urine sample (upper part) and mass spectrum of aniline (lower part)

Table 1 Reported cases of survival after oral aniline ingestion

Case, ref., age, sex	Ingested dose of aniline, circumstances	Met-HB (hours after ingestion)	Symptoms /⇒ Therapy (hours after ingestion)	Plasma aniline in mg/l
1. [7] 22, male	small amount, accidentally ingested by a mechanic (octane booster for a car)	(Initial) n.d. (15) 19% (74) 9%	next hour drowsy, cyanotic ⇒ 2 × MB, (64) cyanosis diminished slowly, (74) Heinz-body hemolysis, jaundice ⇒ blood transfusion required on day 6	n.d.
2. [12] 4.5, female	one teaspoon as "cough-mixture"	(1) 68% (10) 47% (13) 77% (≈ 16) 14%	vomiting, deep cyanosis ⇒ GL, Cat, 2 × MB within 5 h (13) ⇒ MB+ET decorticate posturing, discharge on day 5	n.d.
3. [9] 32, male	30–60 ml accidentally ingested from a soft drink container	(1) >70% (?) 34% (18) 45% (20) 24%	⇒ Card Res, MB, GL, Ac, Cat, AA ⇒ MB, AA ⇒ MB requirement of red cells for 5 days	n.d.
4. [10] 21, female	80 ml ingested patient under psychiatric care	(?) 50.3%	deep cyanosis, coma, shock, generalized seizure ⇒ MB, 1 l blood exchange ⇒ MB, hemodialysis, during the next 24 h patient regained consciousness	25.2 (aniline like compounds) [1]
5. [6] 19, male	5–20 ml (aniline, nitrobenzene) ingested by a student while using a pipette	(2) > 65% (11) 25%	patient unconscious but responded to pain stimuli, lips, tongue blue/black, tachypnea, dyspnea, aspiration of vomit ⇒ GL, MB, AA, ET, day7 hemolytic anemia ⇒ blood transfusion	
6. this case	twice administration by the husband (one small, one larger amount) in coffee	(3.5) 35% (11) 4.3%	strong headache, collapse, cyanosis, ⇒ tolonium chloride, AA, discharge day 3	aniline 0.13 acetanilide 0.79 acetaminophen 2.3

Met-Hb methemoglobin level, *MB* methylene blue administration, *Card Res*, cardiopulmonary resuscitation, *GL* gastric lavage, *AC* activated charcoal, *AA* ascorbic acid, *ET* exchange transfusion, *Cat* cathartic, *n.d.* not determined

Discussion

The husband confessed that he had twice put some aniline into the coffee, once with a small amount and the next day a larger amount which he administered from a bottle with disposable syringes and three 1 ml syringes which were contaminated with aniline were found in the garbage can. A significant elevation in methemoglobin levels in adult volunteers has been reported after oral administration of 25 mg aniline [8]. Severe cases of aniline intoxication were reported after ingestion of a few milliliters and as little as 6 g has been suggested as a lethal dose, but larger amounts have been tolerated [4]. The estimated mean lethal oral dose is between 15 g to 30 g pure aniline [4]. Two facts make it unreliable to correlate ingested doses of a substance with the symptoms of toxicity. When estimating the toxicity of a compound by a given ingested amount, there is always a problem with the accuracy of the statement given by the intoxicated person. Another problem is the additional feature of early vomiting concomitant with many cases of intoxication. This makes it very difficult to estimate how much of a given substance was actually absorbed. Therefore it is much better to determine blood levels as a parameter which in general show a better correlation to symptoms.

Reports of acute aniline poisoning due to the pure chemical are rare. Table 1 gives a list of the few documented cases where pure aniline was orally ingested together with the ingested doses, methemoglobin levels, symptoms and therapy. In the case reported here, the blood and urine levels of aniline and its metabolites were determined and could be approximately correlated to the methemoglobin level. It is well known that the toxicity of aniline is characterized by the formation of methemoglobin and is not due to a direct effect of aniline but to a metabolite. N-oxidation of aniline leads to PHA which is unstable and is rapidly oxidized to nitrosobenzene in the presence of oxygen and methemoglobin is formed as a by-product [12]. Nitrosobenzene is reduced back to PHA in erythrocytes by a NADPH-dependent diaphorase and by means of this catalytic cycle, one molecule of PHA can produce many molecules of methemoglobin [10]. A fast metabolic inactivation of aniline may therefore protect against toxic effects.

Individual differences in the response to aniline must also be considered. In addition to acetanilide the main metabolite detected in blood as well as in the urine sample collected 11 h after ingestion was acetaminophen. Although acetaminophen has never been reported before in humans after aniline ingestion, its formation was to be expected. The studies of Noguchi et al. [13] showed that p-aminophenol, acetanilide and acetaminophen were detectable after addition of aniline to primary liver cell cultures from rats. The major metabolic pathway for aniline in humans is N-acetylation by N-acetyltransferases [16] and ring hydroxylation by the cytochrome P450 system, namely the isoenzymes cytochrome P450 IIE1 and IA2 [17] followed by sulfate and glucuronide conjugation.

Differences in metabolic detoxication of active compounds may account for some of the interindividual variations in susceptibility to aniline. In the case of glucose-6-phosphate dehydrogenase or glutathione reductase deficiency, the reduction of methemoglobin is delayed result-

ing in a higher toxicity of aniline. This also applies to persons with a low N-acetylation capacity by which the bioavailability of aniline will be higher. Examination of urine samples from 63 industrial workers who were exposed to 4,4-methylendianiline showed that acetylation is the most important detoxication route for 4,4-methylendianiline [15]. The individual ratio of the monoacetylated compound to total methylenedianiline varied widely from 0% to 100% which was interpreted by the authors as due to the acetylator phenotypes. The extent of acetylation might have played an important role in this case of aniline intoxication. Keeping in mind the amount ingested, the relatively mild symptoms and the high concentrations of acetanilide and acetaminophen in comparison with p-aminophenol, it might be an explanation that the fast acetylation of aniline and p-aminophenol (with subsequent conjugation) protected the woman from a more severe course.

The pathologist should be aware that the reason for finding contemporary high methemoglobin levels and low concentrations of paracetamol in the blood of a corpse could be caused by a fatal aniline intoxication. This case shows that even very low concentrations of aniline can produce a severe intoxication and the detection of such low concentrations will cause difficulties in routine GC or GC/MS screening. An important criterion for differential diagnosis would be the detection of acetanilide as this can only be the result of aniline metabolism. It reaches higher concentrations than aniline and has better chromatographic properties.

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