Case Report

Fatal Acute Poisoning by Bentazon

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Abstract

A case of fatal suicidal bentazon poisoning is presented along with a description of the different analytical methods involved. A 56-year-old farmer was examined by the family doctor 1 h after voluntarily ingesting 500 mL of FIGHTER® (bentazon, 480 g/L water). He presented a Glasgow score of 15, polypnea, diarrhea, and vomiting. During transport by ambulance to the hospital, he tossed, sweated, and suddenly presented breathing difficulty followed by heart failure. Tracheal intubation was impossible (H1.5) despite use of different diameter cannulas because of extreme general muscle rigidity. All attempts at resuscitation failed, and the patient died within 2 h postingestion. Blood and urine samples were taken just before death. General basic and neutral drug screening by high-performance liquid chromatography–diode-array detection and gas chromatography–nitrogen-phosphorus detection showed no strychnine or other drugs or toxics except for citalopram (< 0.1 mg/L) and bentazon, but this weak acidic molecule (pK_a 3.3) was badly extracted in alkaline conditions. Plasma and urine levels, measured after acidic extraction, protein precipitation, or simple dilution, were 1500 and 1000 mg/L, respectively. Bentazon (M.W. 240) was confirmed by its basic mass spectrum (ESI⁻, m/z 239, 197, 175, 132) or by that of methylated derivative (EI⁺, m/z 254, 212, 175). An hydroxylated metabolite (ESI⁻, m/z 255, 213, 191, 148 ; EI⁺, m/z 284, 242, 163) and the N1-glucuronide conjugate of bentazon (ESI⁻, m/z 415, 239) were also detected in urine. (Quantitation ions are underlined.) This first case of bentazon poisoning with available analytical data revealed the high toxicity of this compound after large dose ingestion with early and heavy symptoms such as muscle rigidity probably related to muscular toxicity. Comparison with another nonfatal case and with toxicological data on animals is discussed.

Introduction

Bentazon or 3-isopropyl-(1H)- 2,1,3-benzothiadiazin-4-one 2,2-dioxide (Figure 1) is a selective contact herbicide particularly used in growing cereals such as rice or corn. It is a polar, weakly acidic pesticide (pK_a 3.3) available in France since the 1980s either in single formulation (Basagran®, FIGHTER) or in association with other herbicides such as chlorophenoxyacids (dichlorprop), atrazine, or bromoxynil. Bentazon inhibits

carbon dioxide fixation in sensible plants and thus halts photosynthesis (1,2).

This pesticide is considered to be slightly toxic for mammals with acute oral toxicity (LD_{50}) of 1100 mg/kg in rats but only 400 and 500 mg/kg in mice and cats, respectively. Only one case of acute poisoning was reported in the literature (3). Shortly after ingestion of 300 mL (132 g in water) Basagran solution, the patient presented with vomiting, fever, sweating, drowsiness, and pipelike muscle rigidity. After gastric lavage and activated charcoal, a bromocryptine treatment was instituted (2.5 mg t.i.d.) for 3 days. Rhabdomyolysis, already installed on day 1 (creatine kinase 1009 U/L), increased on day 2 (4600 U/L) and disappeared on day 22 when he was discharged. No toxicological data were given.

In the fatal case described here, bentazon was searched for in plasma and urine by different analytical approaches such as high-performance liquid chromatography–diode-array detection (HPLC–DAD) with routine toxicological screening (4) or another HPLC–ultraviolet detection (UV) method used for chlorophenoxyacids. Confirmation of bentazon and identification of two metabolites were also undertaken by gas chromatography–mass spectrometry (GC–MS) and by liquid chromatography–mass spectrometry either in single (HPLC–MS) or tandem (HPLC–MS–MS) mode.

Case History

A 56-year-old farmer, who was known to be depressive, ingested 500 mL FIGHTER (480 g/L in water). A first examination by the family doctor (45 min later) showed a polypnea, a
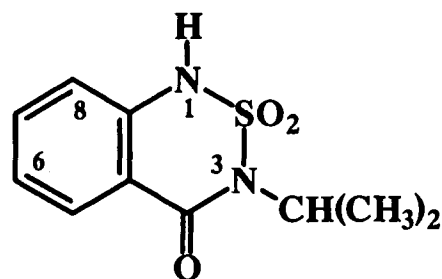

Figure 1. Chemical structure of bentazon.

Glasgow score of 15, 160/80 mm Hg systolic arterial pressure, and a 120/min heart rate. The patient already exhibited some vomiting and diarrhea. Thirty minutes later, during transport to the hospital, he was agitated, sweating, had a new diarrheic episode, and suddenly presented difficulty in breathing and lost consciousness. Five minutes later, cardiac arrest and muscle rigidity occurred. Heart massage was performed until arrival at the emergency unit (SAMU) 7 min later. The patient presented a cardiorespiratory arrest with stoney rigidity, cyanosis, and unreactive bilateral mydriasis. External heart massage and mask ventilation under 100% FiO_2 were unsuccessful. Tracheal intubation was impossible despite the use of different cannulas by either oral or nasal route. The ECG was flat, and electric stimulation before and after adrenaline intracardiac injection gave no result. The patient died 2 h after ingestion of the herbicide, and no autopsy was performed.

Materials and Methods

Two biological samples (peripheral blood and urine) were taken at the time of death. Initial analyses (alcohol, benzodiazepines, and tricyclic antidepressants) were completed with screening procedures by HPLC–DAD (4) and GC–NPD (5) and also by specific analyses (chloralose, cholinesterases, phenoxyacidic, and herbicides). Further investigation was done by MS.

Chemicals

Diethylether (Merck, Darmstadt, Germany), methanol (Prolabo, Val de Reuil, France) and potassium dihydrogenphosphate (Prolabo) were analytical grade and ammonium formiate (Merck) and acetonitrile (Carlo Erba, Milan, Italy) were chromatographic grade. Bentazon (B) was obtained from PRO-MOCHEM (Molsheim, France) and 2-methyl-4-chloro-phenoxyacetic acid (MCPA), used as internal standard from Aldrich Chemical Company (Lyon, France). 6-Hydroxy-bentazon (6-OHB) was a gift from BASF France Laboratories (Levallois, France). Stock solutions were 1 g/L in methanol or acetonitrile (MCPA) and were kept at 4°C.

Apparatus

Four different chromatographic systems were used.

HPLC–DAD was performed with an HP 1090 apparatus (Hewlett-Packard, Les Ulis, France). The reversed-phase chromatography column was a Hypersil ODS 5 μm (100 × 2.1-mm i.d.) equipped with a cartridge precolumn (20 × 2.1-mm i.d.). The mobile phase was a 20mM phosphate buffer(+ triethylamine 0.5 mL/L)/acetonitrile mixture in gradient elution mode as previously described (4), and the flow rate was 400 μL/min.

HPLC–ultraviolet detection (UV) was also done with a specific procedure for the analysis of chlorophenoxyacid herbicides using a mixed system with a Constametric pump and a Spectromonitor III UV detector, a WISP 717 Waters injector (Millipore, Molsheim, France), and an HP 3395 integrator. The chromatographic analysis was performed on a Select B Lichrospher 5-μm column (100 × 4.0-mm i.d.) equipped with precolumn (4 × 4-mm i.d.). The mobile phase was a mixture of ammonium

formiate (2mM) and formic acid (0.1%) containing 1.5 mL triethylamine per liter and acetonitrile (70:30, v/v). The flow rate was 1.5 mL/min, and the detection wavelength was 225 nm.

GC–MS analysis was performed on an Automass system (Unicam, Meylan, France) with a 25-m (0.32-mm i.d., 0,17-μm film thickness) Ultra-2 HP column. The GC conditions were as follows: helium carrier gas (6 p.s.i.), splitless mode (1 min), injector temperature 275°C, oven temperature programmed from 60°C (initial time 1 min) to 170°C at 30°C/min and then up to 240°C at 10°C/min (final time 0.5 min). The MS conditions were as follows: full scan (m/z 40 to 500) mode, electron impact (EI) ionization with a 70 eV energy, and ion source and interface temperatures were 150°C and 250°C, respectively.

LC–MS or LC–MS–MS was done with an API 300 Perkin-Elmer Sciex (Thornhill, ON, Canada) system with a "turbo-ionspray" atmospheric pressure ionization source. The separation was done on a Spheri5 RP8S (100 × 2.1-mm i.d.) column (Applied Biosystems, Courtaboeuf, France) with a flow rate of 400 μL/min without postcolumn split. The mobile phase was ammonium formiate buffer (2mM)-formic acid (0.1%) and acetonitrile in isocratic conditions (60:40, v/v). The main parameters were as follows: the electrospray voltage adjusted at 5 kV in a negative mode, the orifice ring (OR) at –31 V, the source temperature at 450°C and the nebulization gas (air) and curtain gas (nitrogen) flow rates 1.1 L/min and 0.9 L/min, respectively. Scans were made from m/z 90 to m/z 450.

LC–MS–MS was done with a low OR voltage (–31 V) to avoid fragmentation in the ionization source. In the collision cell, a fragmentation energy of 20 eV was used and the gas (nitrogen) flow-rate was 0.3 L/min. Two different acquisition modes were evaluated (product ion scan and precursor ion scan) in order to detect possible metabolites. The effects of all the instrumental parameters known to affect fragmentation were not evaluated.

Sample preparation

Condition 1. Liquid–liquid alkaline extraction was done as previously described (4). Liquid–liquid acidic extraction was done by mixing, for 10 min, 5 mL dichloromethane, 500 μL plasma or urine, 25 μL internal standard solution, and 500 μL sodium acetate (0.2M pH 4.0) buffer. The organic layer was evaporated to dryness at 50°C under nitrogen, and the residues were dissolved in 50 μL methanol and 20 μL water. Ten microliters was injected.

Conditions 2 and 4. The plasma and urine (100 μL) were analyzed after simple protein precipitation by adding 100 μL acetonitrile containing MCPA (50 mg/L) as internal standard. Then the supernatant was half-diluted with water, and 25 μL was injected. Quantitation of B was done with UV (225 nm) conditions on plasma and urine samples after dilution (1/10e or more) in a free-drug plasma, using internal standard peak-area ratio method and a six-point calibration curve (5, 10, 20, 30, 50, and 100 mg/L).

Condition 3. The plasma and urine were prepared as previously described for the barbiturate screening (6): methohexital (25 μL of a 0.1 mg/mL methanolic solution) was added as internal standard to 100 μL sample, 250 μL methanol/water (90:10) solution saturated with potassium carbonate, and 10 μL dimethylsulfate. The stoppered polypropylene tubes were heated

at 50°C for 5 min and then, after the addition of 250 µL of sodium acetate (0.2M, pH 6.0) buffer, extracted by 1 mL hexane. One microliter of the upper layer was injected. Quantitation of B was done as described in Condition 2 by monitoring the *m/z* 212 ion for B versus *m/z* 235 ion for IS.

Results

HPLC–DAD after alkaline extraction on plasma sample showed citalopram with a low level (< 0.1 mg/L) and a rapidly eluted peak (retention time, RT = 1.5 min) having a spectrum with two maxima absorbance wavelengths (225 and 335 nm, Figure 2). This compound, not initially reported in our library of 500 drugs and 150 pesticides, was further identified by comparison with the pure bentazon. Although this product is a weak acid, it was observed after alkaline extraction, which is suggestive of high levels. Under acidic extraction conditions, the bentazon peak was highly enhanced. Other analyses such as alcohols, immunodosages, chloralose, and strychnine were negative, and cholinesterase activity was normal.

Under the phenoxyacid chromatographic conditions, bentazon was eluted with an earlier RT than MCPA (2 versus 3.6 min) and RTs of analogue compounds are presented in Table I.
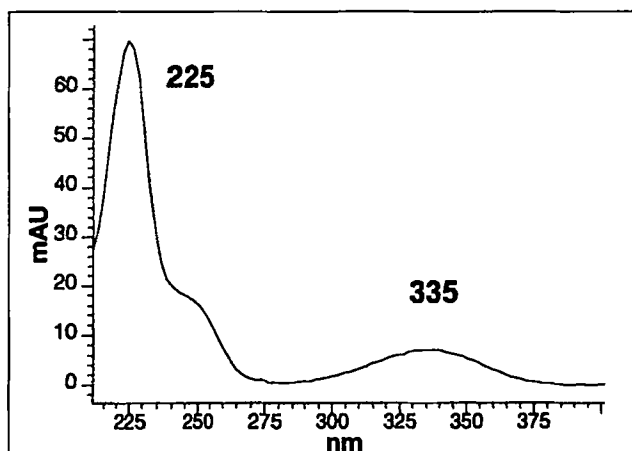

Figure 2. UV spectrum of bentazon.

Table I. Retention Times (RT) of Bentazon and Phenoxyacid Herbicides on Lichrospher Select B with Formiate Buffer/Acetonitrile Mixture (70:30) as Described in Condition 2

Compound	RT (min)
Bentazon	2
2,4-Dichlorophenoxyacetic acid (2,4D)	3.3
2-Methyl 4-chlorophenoxyacetic acid (MCPA)	3.6
2,4-Dichlorophenoxypropionic acid (2,4DCP)	5.2
2,4,5-Trichlorophenoxyacetic acid (2,4,5T)	5.5
2-Methyl 4-chlorophenoxypropionic acid (MCPP)	5.8
Ioxynil	6.9
2-Methyl 4-chlorophenoxybutyric acid (MCPB)	12.8
2,4-Dichlorophenoxybutyric acd (2,4DB)	13.1

GC–MS analysis after methylation confirmed the identification of bentazon (Table II) with a parent ion (*m/z* 254) and a typical fragment (M-42) corresponding to the loss of the isopropyl radical. This analysis revealed the presence of another peak with a higher RT (9.2 min) and a low (1/50) intensity in urine. The M+30 ion and the higher RT suggested a possible hydroxylated compound with double methylation.

HPLC–MS analysis was in agreement with these results: bentazon (RT = 2.2 min) was identified by its M-1 (*m/z* 239) ion.

Table II. Retention Times (RT) and Spectral Data of Bentazon and Hydroxy-Bentazon Methyl Derivatives after Positive Ionization in GC–MS as Described in the Apparatus Section*

Compound	RT (min)	M+	Fragment ions
Hydroxy-bentazon	9.2	284 (20)	<u>242</u>, 163 (80)
Bentazon	7.5	254 (12)	<u>212</u>, 175 (20), 133 (18), 105 (55)

* Base peak is underlined, and the relative abundance is in parenthesis.

Table III. Retention Times (RT) and Spectral Data of Bentazon, N1-Glucuronide, and Hydroxylated Metabolites Obtained from Urine Sample after Negative Ionization in HPLC–MS–MS as Described in the Apparatus Section*

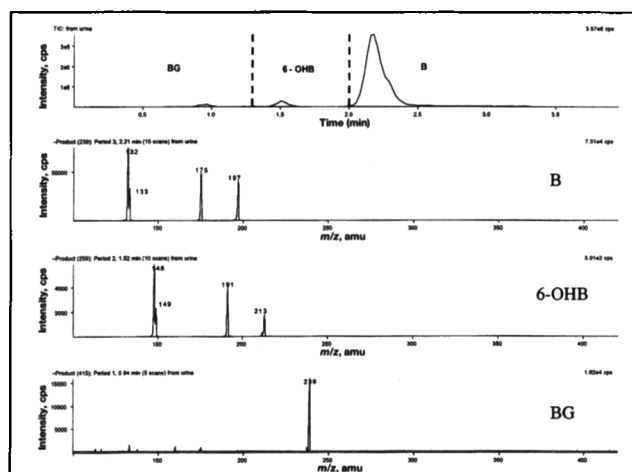| Compound	RT (min)	(M-H)⁻	Fragment-ions
Bentazon glucuronide	0.95	415	<u>239</u>
Hydroxy-bentazon	1.5	255	213, 191, <u>148</u>, 149
Bentazon	2.2	239	197, 175, <u>132</u>, 133

* Base peak is underlined.

Figure 3. HPLC–MS–MS chromatogram (upper part) from urine and corresponding product ion spectra (other parts) with negative ionization mode (OR = –31V) with analysis performed in three experiments: 0–1.3 min, glucuronide searching (BG spectrum) by acquisition-fragmentation of *m/z* 415 ion; 1.3–2 min, hydroxy metabolite searching (6-OHB) *m/z* 255 ion; 2–4 min, bentazon searching (B) *m/z* 239 ion.

The hydroxylated metabolite with a lower RT (1.5 min) was confirmed by its M+16 (m/z 255) ion compared to the pseudomolecular ion. Another peak with a m/z 239 ion was also detected with a shorter RT (0.95 min). Precursor ion scan of this m/z 239 ion gave a pseudomolecular m/z 415 ion. The fragmentations of these different compounds obtained in product-ion scan mode were given in Table III and Figure 3. The m/z 197, 175, 132, and 133 ions were assigned to the loss of the isopropyl radical (M-42), the SO_2 group (M-64), or their sum from bentazon or bentazon minus proton ions, respectively. A shift of +16 was observed for all fragment ions of the metabolite. This compound was further confirmed as 6-OHB by comparison with the pure product that gives identical RT and mass spectra both in GC and HPLC. The other metabolite with a theoretical molecular weight of 416 gave a m/z 239 fragment ion: this loss of 176 and the shorter RT could be related to an N1-glucuronide metabolite of B.

The six-point calibration curve showed a good linearity both after HPLC–UV and protein precipitation (B area ratio = 0.07 [B concentration] + 0.02; r^2 = 0.999) and after GC–MS and derivatization (B area ratio = 0.033 [B concentration] – 0.09; r^2 = 0.999). Mean plasma level of B was 1500 mg/L (1450 or 1550 mg/L, respectively), and mean urine level was 1000 mg/L (950 and 1050 mg/L, respectively). No quantitation of OHB was done.

Discussion

Available analytical data on bentazon are essentially composed of trace analyses in environmental and/or river waters (7–10) and were performed either by HPLC–DAD or HPLC–MS (or MS–MS). The negative ionization mode gave the best results because of the weak acidic property of B. Our results are in good agreement with the mass fragmentation results already described. No data were found on GC–MS analysis, but bentazon and its hydroxylated metabolite are easily detected as methylated derivatives. The detection limits of our methods were not evaluated, but levels as low as 1 mg/L were well detected with a biological sample of only 100 μL. Laboratories involved in toxicological analyses on biological samples could easily search for bentazon with an HPLC–UV only, in the same conditions as for phenoxy-acid herbicides without an absolute need for UV or MS data.

The bentazon metabolism by hydroxylation can give two possible derivatives on either the 6 or 8 positions of the phenyl ring. These two metabolites have the same chromatographic characteristics, the same mass spectra, and identical fragmentations (7). Thus, they cannot easily be differentiated. 6-OHB only was obtained from BASF France Laboratories. Hence, a hydroxylation in the 8 position cannot be excluded, but an in vitro study (11) indicated that 6-OHB was the major phase I metabolite in corn shoots and that this hydroxylation is under cytochrome P450 control. However, the two hydroxylated metabolites were detected in river water (7). A metabolism study in rats using [^{14}C] bentazon and thin-layer chromatography described a major urine compound identified as unchanged bentazon and two minor metabolites, one of them

possibly being the N-1 glucuronide derivative (12). Despite a probable metabolism of bentazone by cytochrome P450, acute experiments in rats showed that the toxicity of oral bentazon remains unchanged by the barbiturate pretreatment and that bentazon elicits lethal effects that are not related to either gender or preliminary induction of the liver's pharmacometabolic rate (13). Toxicokinetic studies in the rat with [^{14}C] bentazon revealed that it was rapidly absorbed and mostly excreted in the 24-h urine, that only traces were present in the bile, and that no radioactivity was detected in the brain or spinal cord of animals (12).

To our knowledge, this is the first reported case of fatal intoxication by bentazon, and the death could be explained by the supposedly large dose ingested and the very high levels observed in biological samples 2 h after ingestion. This is in agreement with toxicokinetic data in animals. Clinical symptoms of acute oral poisoning in pheasants and rabbits occured predominantly in the respiratory system (14). Shallow accelerated breathing, dyspnea, central nervous system depression, pronounced increase of body temperature, and rapid onset of rigor mortis were observed, and the animals died as a result of asphyxia (14). The symptoms in the case presented are identical to those in animals. In addition, fever, drowsiness, pipelike muscle rigidity and rhabdomyolysis were reported in another case of human poisoning (3). The mechanism of the toxicity, hypertonia and hyperthermia, is unknown. A muscular toxicity could be suspected. Other authors reported a toxicity mimicking neuroleptic malignant syndrome, but central nervous toxicity is unlikely as bentazon does not cross the blood-brain barrier according to experimental data.

Treatment of bentazon poisoning is symptomatic, and respiratory support is the main challenge. Tracheal intubation may be impossible because of hypertonia, but the effect of benzodiazepine or competitive neuromuscular blocking agents remains to be evaluated.

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