CASE REPORT

Albert D. Fraser,[1] *Ph.D.; Wallace MacNeil,*[2] *B.Sc.; and Arthur F. Isner*[2]

Toxicological Analysis of a Fatal Baclofen (Lioresal) Ingestion

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ABSTRACT: A fatality following ingestion of the drug baclofen (Lioresal®) is described. Baclofen was identified in urine by gas chromatography/mass spectrometry. After derivatization with trinitrobenzene sulfonic acid, baclofen was quantitated in serum and urine by high-performance liquid chromatography. The concentration of baclofen was 17 mg/L in serum and 760 mg/L in urine collected approximately 12 h after the overdose. To our knowledge, this is only the second reported fatality involving a baclofen overdose. The previous case did not include quantitation of baclofen in any biological fluid.

KEYWORDS: toxicology, baclofen, death, chromatographic analysis, drug overdoses

Baclofen (Lioresal®) is an analog of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). This compound (Fig. 1) is more lipophilic than GABA and can readily penetrate the blood-brain barrier. There is no evidence that baclofen's action is related to binding or to some other effect on a GABA receptor or receptors. Baclofen is known to inhibit both monosynaptic and polysynaptic reflexes at the spinal level [*1–3*].

Baclofen is prescribed for treatment of spasticity resulting from multiple sclerosis and for the treatment of various spinal cord injuries [*1*]. Plasma concentrations increase in a proportional manner with increasing dosage. With single doses of 15 to 90 mg, trough plasma baclofen concentrations ranged from 100 to 400 ng/mL. Toxic blood levels have been reported to range from 1100 to 3500 ng/mL [*4–6*]. Baclofen is 30% bound to plasma proteins, and 85% of a dose is excreted unchanged in the urine. The remaining 15% of a dose is deaminated to β-(*p*-chlorophenyl)-γ-hydroxybutyric acid [*5*].

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[1]Toxicologist, Toxicology Laboratory, Victoria General Hospital, and Department of Pathology, Dalhousie University, Halifax, Nova Scotia, Canada.

[2]Supervisor and technologist, respectively, Toxicology Laboratory, Victoria General Hospital, Halifax, Nova Scotia, Canada.

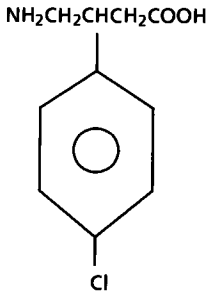

FIG. 1—*Chemical structure of baclofen.*

Several reports summarize nonfatal intoxications with baclofen [*1–7*]. From 1975 through 1985, there were 11 reported cases of baclofen overdose. The amount of drug ingested ranged from 420 to 1520 mg. In the only reported death due to baclofen poisoning, a 51-year-old female died after ingesting 1250 to 1500 mg of baclofen. Coma, muscle flaccidity, respiratory depression, and hyporeflexia are all consistent clinical features of baclofen poisoning. This report documents analytical findings in serum and urine of baclofen in a single overdose case.

Case History

This 30-year-old male had been a quadriplegic following a motor vehicle accident at age 27. Investigation at his home revealed that he had received 50 to 90 tablets (20 mg) of baclofen (Lioresal) from his caretaker. His normal daily dose was 80 mg. Approximately 12 h later, he was found in a coma and taken to a hospital. He was taken to an intensive care unit and died 5 days after admission.

Analysis of serum and urine for baclofen and other drugs was performed on specimens collected shortly after his admission to the hospital.

Toxicologic Analysis

Standards and Reagents

The acetonitrile and methanol used were high-performance liquid chromatography (HPLC)-grade and glass distilled (Caledon Laboratories, Ltd., Georgetown, Ontario, Canada). The baclofen standard was obtained from Ciba Geigy Canada Ltd., Mississauga, Ontario. The internal standard (gabapentin) was obtained from Parke-Davis Pharmaceutical Research Division, Ann Arbor, Michigan. Sodium tetraborate and acetone were obtained from Mallinckrodt Chemicals, Montreal, Quebec. The derivatizing reagent 2,4,6-trinitrobenzene sulfonic acid (TNBSA) was purchased from Pierce Chemical Co., Rockford, Illinois.

Thin-Layer Chromatography

Drug screening on urine was performed by thin-layer chromatography (TLC) as has been described previously [*8–9*].

High-Performance Liquid Chromatography

Liquid chromatography was performed on a Model 740 solvent delivery system by Spectra Physics, an SF 770 variable wavelength ultraviolet detector by Schoeffel Instruments, and an Omniscribe recorder by Houston Instruments (all obtained from TMA Associates, Halifax, Nova Scotia, Canada). Analysis was performed at ambient temperature using a 250 by 4.6-mm RP-8 column (5-μm particle size) obtained from Brownlee Laboratories, Santa Clara, California.

The detector wavelength was set at 340 nm. The mobile phase was a buffer solution consisting of a 1:1 mixture of acetonitrile and 0.08*M* acetate buffer (pH 4.7).

Gabapentin [1-(aminomethyl)cyclohexane acetic acid] was used as the internal standard. A stock solution of baclofen was prepared by weighing 10 mg of the drug and dissolving it in 10 mL of water. Working standards were prepared in drug-free bovine serum to give final concentrations ranging from 5 to 20 mg/L. The internal standard was prepared by dissolving 10 mg of gabapentin in 10 mL of acetone. This solution was further diluted with acetone to give a final concentration of 20 mg/L.

Two hundred and fifty milligrams of TNBSA was dissolved in 25 mL of distilled water. Sodium tetraborate (0.05*M*) was prepared by weighing 0.48 g of the powder and dissolving it in 25 mL of distilled water.

A 0.5-mL aliquot of serum or standard was placed in a 1.5-mL microcentrifuge tube, and proteins were precipitated by the addition of 0.5 mL of acetone containing the internal standard. After mixing and centrifugation, a 0.5-mL aliquot of the supernatant was evaporated to dryness under nitrogen gas (N_2) in another tube at 50°C in a heating block. The residue was then dissolved in 50 μL of 1% TNBSA and 50 μL of 0.05*M* sodium tetraborate. After being mixed, the solution was allowed to stand at room temperature for 60 min for derivatization to occur. Baclofen and the internal standard were purified by the addition of 20 μL of 5*M* acetic acid prior to the mixing and centrifugation. The supernatant was decanted as waste and the precipitate (containing baclofen and the internal standard) was dissolved in 100 μL of the mobile phase. This mixture was centrifuged again before the supernatant was injected onto the HPLC column.

The urine specimen was diluted 1:50 prior to the addition of the internal standard and analyzed directly after the addition of acetic acid.

Peak height ratios were used to calculate drug concentrations. The baclofen procedure is a modification of a previously published procedure for gabapentin in serum [*10*].

Gas Chromatography/Mass Spectrometry

Gas chromatography/mass spectrometry (GC-MS) identification of baclofen was performed on a Hewlett-Packard 5890 gas chromatograph operated in the split injection mode (10:1 ratio) attached to an HP 5970B mass selective detector. A DB-1 column (15 m by 0.26 mm in inside diameter) was operated in a temperature programing mode from 100 to 300°C (held at 100°C for 1.5 min and raised 15°C/min to 300°C) and held at 300°C for 3 min.

For GC-MS analysis, a 100-μL urine specimen was evaporated to dryness and taken up in 100 μL of methanol for direct injection onto the column without derivatization.

Results

The concentration of baclofen in serum and urine was 17 and 760 mg/L, respectively. A chromatogram for the serum extract is found in Fig. 2.

Qualitative identification of baclofen in urine was performed by GC-MS. A total ion chromatogram (TIC) for the urine extract is found in Fig. 3. The mass spectrum for a baclofen standard and the urine extract are found in Fig. 4.

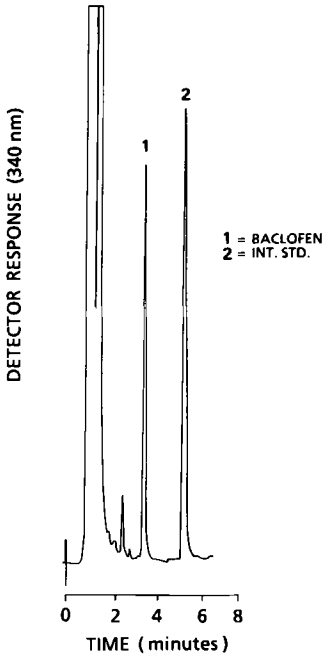

FIG. 2—*High-performance liquid chromatogram of serum extract: (1) baclofen 17 mg/L and (2) gabapentin internal standard.*

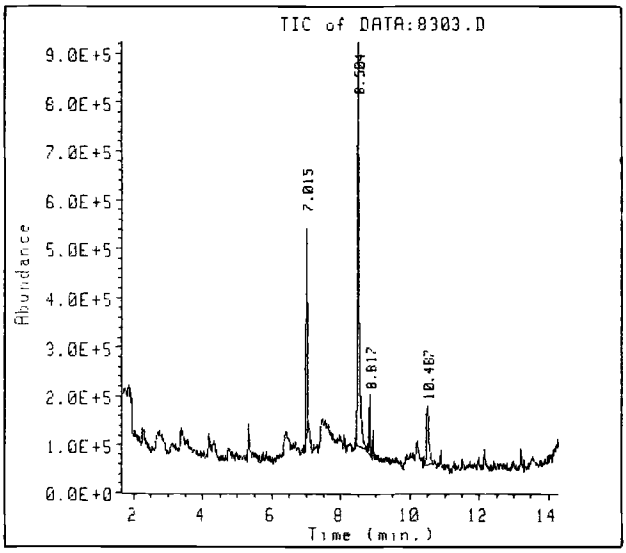

FIG. 3—*Total ion chromatogram of the urine extract containing baclofen (retention time, 8.504 min).*

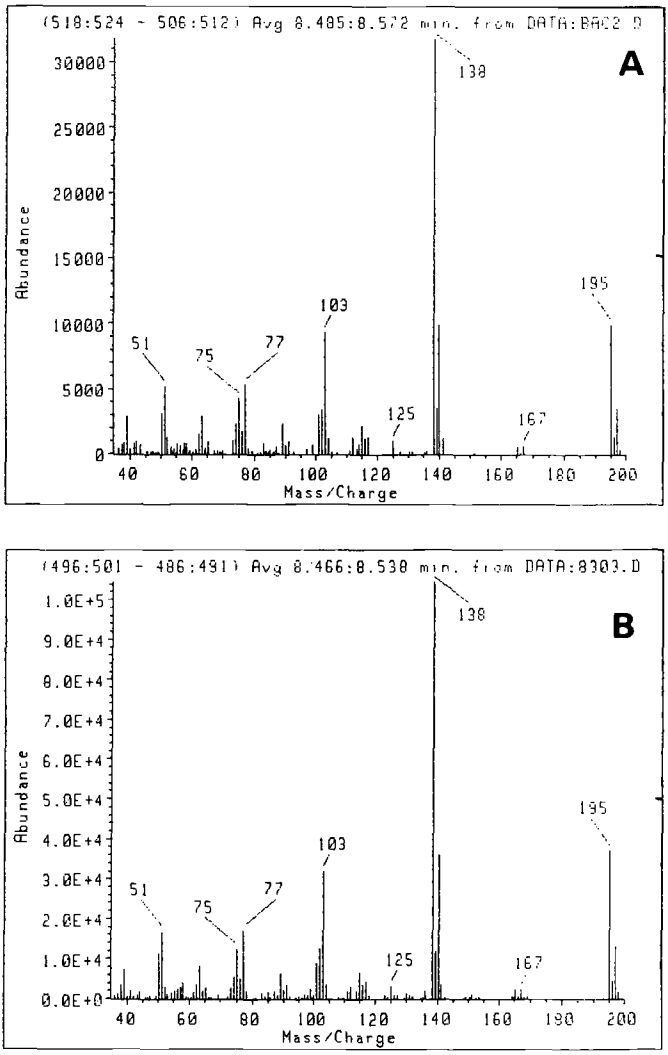

FIG. 4—*Mass spectrum of baclofen pure standard* (A) *and urine extract* (B).

The HPLC method for baclofen was linear from 5 to 20 mg/L. The limit of detection was 0.8 mg/L, based on a signal/noise ratio of 3:1 at that concentration.

The routine urine drug screening procedure did not detect baclofen or any other drug.

Discussion

In the first report on baclofen analysis, the analysis was performed by gas chromatography after derivatization to the butyl ester [*11*]. The butyl esters were *N*-acylated with heptafluorobutyrylimidazole prior to being injected onto a packed column of 3% OV-225 on Chromosorb W-HP.

Harrison [*12*] described an HPLC method for baclofen quantitation without derivatization. Baclofen was extracted from plasma onto C_{18} Bond-Elute™ columns prior to

analysis on a RP-18 column with detection at 220 nm. Recovery of baclofen averaged 39% at 50 ng/mL and 46% at 1000 ng/mL. The chromatograms had several large peaks not removed in the extraction procedure, and the authors did not include an internal standard in their method.

Rustem [*13*] published another HPLC method for quantitation of baclofen in plasma with ultraviolet (UV) absorption detection. The author commented that because of the amino acid structure of baclofen, it was difficult to develop an analytical method that was specific for baclofen. He also stated that monitoring baclofen by UV absorption was a problem because of its poor UV absorptivity. Despite these limitations, he described a method using UV detection at 220 nm, analysis without the use of an internal standard, and chromatograms showing several potential interfering peaks eluting near baclofen.

The decision to use the TNBSA derivative and multiple extraction and purification steps was based on the previously published method for gabapentin, another drug that is a GABA analog. Since the derivatization reaction also reacted with endogenous substances such as amino acids and peptides, multiple purification steps were required to remove these interfering peaks invariably seen on the chromatogram.

The identification of baclofen was based on GC-MS analysis of a urine extract. No spots for baclofen were observed by thin-layer chromatography.

Summary

In summary, this report describes the analysis of the GABA analog baclofen (Lioresal) in biological fluids following a massive overdose of baclofen. To our knowledge, this is the first reported baclofen-related fatality that included quantitation of the drug in biological fluids. The decedent died of medical complications secondary to an overdose of baclofen.

References

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Address requests for reprints or additional information to
Dr. A. D. Fraser
Head, Toxicology Laboratory
Victoria General Hospital
1278 Tower Road
Halifax, Nova Scotia
Canada B3H 2Y9