Case Report

Analysis of Metformin in Antemortem Serum and Postmortem Specimens by a Novel HPLC Method and Application to an Intoxication Case

Karla A. Moore, Barry Levine, Jack M. Titus, and David R. Fowler
Office of the Chief Medical Examiner, State of Maryland, 111 Penn Street, Baltimore, Maryland 21201

Abstract

A case of intoxication from the oral hypoglycemic drug metformin is presented. A number of published liquid chromatographic methods were combined to enable a simplified analysis of metformin in both antemortem and postmortem specimens. The method involved direct injection of a protein-free filtrate into the liquid chromatograph. The method was sufficiently sensitive to detect therapeutic use of metformin; no common therapeutic or abused drugs interfered with the assay. In the presented case, the hospital admission serum metformin concentration was 141 mg/L, or approximately two orders of magnitude above therapeutic concentrations. The medical examiner concluded that the cause of death in this case was metformin intoxication, and the manner of death was suicide.

Introduction

Metformin is a biguanide drug used as an oral hypoglycemic agent. It was approved for use in the United States as a treatment of type II diabetes mellitus in 1995. Metformin lowers blood glucose in a number of ways: (1) by decreasing hepatic glucose output, (2) by increasing peripheral glucose utilization, (3) by decreasing fatty acid oxidation, and (4) by increasing glucose turnover. Specifically, it suppresses gluconeogenesis in the liver by potentiating the effects of insulin and by reducing the hepatic extraction of certain substrates such as lactate, as well as by opposing the effects of glucagon. As a result, the presence of insulin is required for drug efficacy (1).

A number of methods have been published for the analysis of metformin in clinical specimens. Freedman et al. (2) published a colorimetric method for the structurally similar biguanide drug phenformin using an alpha-naphthol-diacetyl color reagent. A gas chromatographic-nitrogen phosphorus detection (GC-NPD) method using p-nitrobenzoyl chloride as the derivatizing reagent has also been developed (3). The most common technique used for the analysis of metformin is high-performance liquid chromatography (HPLC) (4-11). Specimen preparation has utilized protein precipitation with trichloroacetic acid or acetonitrile and/or solid-phase extraction. Some methods have employed a precolumn derivatization with p-nitrobenzoyl chloride or tetrabutylammonium hydroxide. Analytical separation has been achieved using either a cation exchange or a reverse-phase column. Wavelengths of detection have usually been 230–240 nm.

The following is a case investigated by the Office of the Chief Medical Examiner, State of Maryland (OCME) in which an intoxication from metformin was suspected from the investigation. Aspects of a number of published HPLC methods were combined to enable a simplified analysis of metformin in both antemortem and postmortem specimens.

Case History

The decedent is a 48-year-old African-American male who had been depressed for several months because of marital problems and an imminent separation from his wife. He had made several previous suicide attempts. His past medical history was significant for type II diabetes for which he had been prescribed the oral hypoglycemic agents metformin, glipizide, and pioglitazone. This was complicated by hypertension, which was being treated with the antihypertensives lisinopril and atenolol, as well as an oral hyperlipidemic agent (simvastatin). Additionally, an antiplatelet agent (clopidogrel) had been prescribed for a previous myocardial infarction (MI), and he had been placed on fluoxetine for depression. At approximately 2200 h on the evening of Day #1, police responded to a call of “a suicide in progress”. Upon arrival at the scene, the wife of the decedent told the officers that the decedent had taken a large quantity of pills with a large amount of alcohol, had cut his wrists, and had locked himself in the house. Officers observed him through the front window further cutting his wrists and starting to cut his throat. They forced the door open at which time the decedent charged the officers with the razor in his hand. When he failed to submit to arrest, he was subdued with “pepper balls” until he became compliant. He was then transported to the regional hospital at 2315 h. A “finger-stick” blood glucose performed by the ambulance
lance crew was 183 mg/dL. At the hospital (0230 h on Day #2), a “finger-stick” blood glucose was 106 mg/dL. As a result of this decreasing value and the history of possibly taking an overdose of multiple oral hypoglycemics, the decedent was started on intravenous (IV) glucose supplementation. Additionally, blood gases and electrolytes drawn on admission were significant for an anion gap of 19 and arterial pH 7.07, consistent with metabolic acidosis. The blood alcohol content was 41 mg/dL. By 0700 h of Day #2, the anion gap had increased to 24 and the arterial pH had decreased to 6.8. His blood glucose was 258 mg/dL because of continued IV supplementation. On the evening of Day #2, his status deteriorated to a coma at which time he was put on respiratory support and dialysis. At 0845 h on Day #3, he “coded” and was pronounced dead at 1050 h.

The autopsy was performed at the OCME on the morning of Day #4. Significant findings included multiple pepper-spray ball wounds consisting of circular abrasions and contusions to both sides of the upper back, nine superficial, scratch-like cutting wounds to the left wrist, two superficial scratch-like cutting wounds to the right wrist, and acute bronchopneumonia with hyaline membrane formation associated with ventilator dependency. Hospital and postmortem specimens were sent to the toxicology laboratory for analysis.

Experimental

Standards
Metformin and phenformin were purchased from Sigma Chemical Co. (St. Louis, MO), and 50-mg/L solutions in water were prepared. Phenformin served as the internal standard in the metformin analysis.

Metformin analysis
To 1 mL calibrator, control, and case fluid or homogenate (1 part tissue and 4 parts water), were added 100 mL of internal standard solution and 1 mL of acetonitrile. After vortex mixing each tube for 30 s, the tubes were centrifuged at 3500 rpm for 10 min, 800 mL of the clear supernatant was transferred to appropriately labeled HPLC injection vials, and 10 mL was injected into the HPLC. Metformin was quantitated using the area ratio of metformin to phenformin in case specimens in comparison to fortified matrix-based specimens at concentrations of 2.5, 5.0, and 10 mg/L. Appropriate dilutions of the case specimens were prepared to ensure quantitation within the standard curve.

Instrumentation
Metformin analysis was performed using a Waters 501 HPLC pump, a 490 E programmable multiwavelength detector, a Waters 715 Ultra WISP autosampler, and a Hewlett-Packard 3396 integrator. Analytical separation was achieved using a Phenomenex “Prodigy 5u” C-18 reversed-phase HPLC column (150 mm × 4.6-mm i.d., 5-μm particle size). The column was maintained at 30°C. The wavelength of detection was 236 nm. The mobile phase was 20:80 acetonitrile/0.01M potassium phosphate, monobasic (KH2PO4) buffer (pH 7.0), ensuring the pH by adjusting with 8M NaOH if necessary. The flow rate was 1 mL/min. Under these conditions, the retention time of metformin was 6.3 min, and the retention time of the internal standard was 8.4 min.

Results and Discussion
Table I lists the performance characteristics of the metformin assay. Table II lists the drugs tested and found not to interfere with the metformin assay.

All postmortem blood specimens were collected in specimen cups. Postmortem heart blood and bile were tested for volatiles and therapeutic and abused drugs. This included volatile testing for methanol, ethanol, acetone, and isopropanol by headspace GC; acid/neural drug testing by GC–NPD; alkaline drug testing by GC–NPD; morphine by radioimmunoassay; and acetaminophen, etchlorvynol, and salicylate by color test. No ethanol or other volatile substances were detected in either specimen. The bile was positive for lidocaine, morphine, and midazolam; no drugs were detected in the postmortem blood. Because the case history suggested an intoxication from drugs not routinely included in this laboratory’s testing, special testing was performed on the Day #1 hospital blood. Specifically, the blood was tested by a reference laboratory for atenolol and for

| Table I. Performance Characteristics of the Metformin Assay (in Blood) |
|------------------|------------------|------------------|
| Limit of quantitation | 0.5 mg/L |
| Linear range (r² > 0.99) | 0.5–20 mg/L |
| Within-run coefficient of variation (at 2.5 mg/L in blood) | 9.2% |

| Table II. List of Drugs Found Not to Interfere with Metformin Assay |
|------------------|------------------|------------------|
| Alprazolam | Clonazepam | Phenmetramide |
| Amitriptyline | Haloperidol | Phenylpropanolamine |
| Amobarbital | Hydrocodone | Phentolamine |
| Amoxapine | Hydroxyzine | Phenotylin |
| Amphetamine | Imipramine | Primidone |
| Atropine | Ketamine | Procainamide |
| Benztpine | Lidocaine | Procaine |
| Brompheniramine | Loxapine | Promethazine |
| Butalbital | Meperidine | Propoxyphene |
| Caffeine | Methadone | Prometheine |
| Chlorpromazine | Methamphetamine | Pseudoephedrine |
| Citalopram | MDA | Pyrilamine |
| Cocaine | MDMA | Quinidine |
| Codeine | Metoclopamid | Quinine |
| Cyclobenzaprine | Minazepine | Thiordizene |
| Desipramine | N-Acetylprocainamide | Trazodone |
| Dextromethorphan | Norfluoxetine | Trifluoperazine |
| Diazepam | Nortripylne | Trimethoprime |
| Dilazem | Oxycodeone | Trimipramine |
| Diphenhydramine | Pentobarbital | Trimethoprim |
| Doxepin | Phencyclidine | Venapamil |
| Doxylamine | Phenobarbital | Zolpidem |
sulfonylurea hypoglycemic drugs. No atenolol was detected in
the blood; glypizide at an approximate concentration of 3.2
mg/L was detected. The OCME toxicology laboratory tested all
available hospital antemortem specimens for metformin. Table
III provides the analytical results.

Two previously published studies indicate a peak serum me-
formin concentration as high as 4.0 mg/L may be reached fol-
lowing the oral administration of a 1500-mg dose of metformin
(12,13). In two cases of metformin intoxication, plasma con-
centrations of 110 and 85 mg/L were reported (14,15). Both in-
dividuals developed a lactic acidosis, a major toxic effect of the
drug, especially with compromised hepatic or renal function. In
the presented case, the Day #1 hospital serum concentration
was above these concentrations and approximately two orders
of magnitude above concentrations seen with therapeutic use.
Moreover, the patient demonstrated a metabolic acidosis that is
consistent with metformin intoxication. As a result, the medical
examiner concluded that the cause of death in this case was
metformin intoxication, and the manner of death was suicide.

The reported elimination half-life of metformin is 4–8 h (15).
The serial serum samples from the hospital indicated a longer
elimination half-life. Because metformin is not metabolized, the
possibility of enzymatic saturation is not an issue. It is likely
that the mechanism of excretion was overloaded, leading to
the increased half-life. One other interesting finding was that
the postmortem blood metformin concentration was higher
than the last two antemortem serum concentrations. There
are several possible explanations for this observation. Post-
mortem redistribution of drugs is a well-documented phe-
nomenon, especially because metformin is a basic drug with a
possibility is that although metformin is not bound to plasma
proteins (15), it may bind to the red blood cells. Finally, the hos-
pital serum was collected in serum separator tubes (SST). It is
a well-recognized phenomenon that many drugs become
trapped in the SST gel, thus significantly reducing their con-
centration in serum versus whole blood (16).

One other unique feature of the postmortem distribution of
metformin in this case was the high concentration of the drug
in the kidney relative to the amount in the liver. This probably
is not surprising given the fact that the drug is cleared primarily
through the kidney.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Concentration (mg/L or mg/kg)</th>
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<tbody>
<tr>
<td>Hospital serum (Day #1)</td>
<td>141</td>
</tr>
<tr>
<td>Hospital serum (Day #2)</td>
<td>62</td>
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<tr>
<td>Hospital serum (Day #3)</td>
<td>45</td>
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<tr>
<td>Heart blood</td>
<td>75</td>
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<tr>
<td>Peripheral (subclavian) blood</td>
<td>77</td>
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<tr>
<td>Bile</td>
<td>271</td>
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<tr>
<td>Liver</td>
<td>146</td>
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<tr>
<td>Kidney</td>
<td>798</td>
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</table>

Table III. Distribution of Metformin in the Presented Case

References

1. N.F. Wiernsperger and C.J. Bailey. The antihyperglycemic effect of
2. L. Freedman, M. Blitz, E. Ginsberg, and S. Zak. Determination of
3. J. Brohon and M. Noel. Determination of metformin in plasma at
therapeutic levels by gas-liquid chromatography using a nitrogen
4. M.S.F. Ross. Determination of metformin in biological fluids by
derivatization followed by high-performance liquid chromatography.
5. B.G. Charles, N.W. Jacobsen, and P.J. Ravenscroft. Rapid liquid-
chromatographic determination of metformin in plasma and urine.
6. L. Benz, P. Marchetti, P. Cecchetti, and R. Navalessi. Determination of
metformin and phenformin in human plasma and urine by re-
verse-phase high-pressure liquid chromatography. J. Chromatogr.
7. J. Keal and A. Somogyi. Rapid and sensitive high-performance
liquid chromatographic assay for metformin in plasma and urine
using ion-pair extraction techniques. J. Chromatogr. 378: 503–508
(1986).
8. R. Huupponen, P. Ojala-Karlsson, J. Routu, and M. Koulu. De-
termination of metformin in plasma by high-performance liquid
9. O. Vesterqvist, F. Nabbie, and B. Swanson. Determination of met-
formin in plasma by high-performance liquid chromatography
10. A.R. Bonfigli, S. Martrini, F. Gregorio, R. Testa, I. Testa, G. De Sio,
and G. Coppa. Determination of plasma metformin by a new
cation-exchange HPLC technique. Ther. Drug Monit. 21: 330–334
(1999).
11. M. Zhang, G.A. Moore, M. Lever, S.J. Gardner, C.M.J. Kirkpatrick,
and E.J. Begg. Rapid and simple high-performance liquid chro-
matographic assay for the determination of metformin in human
of metformin after intravenous and oral administration in man.
and H.F. Woods. Metformin kinetics in healthy subjects and in pa-
14. A. Assan, C. Heuvelin, D. Ganeval, C. Bismuth, J. George,
and R. Girard. Metformin-induced lactic acidosis in the presence of
15. Y. Tasholtis. Disposition of Toxic Drugs and Chemicals in Man, 6th
16. A. Dasgupta, M.A. Yared, and A. Wells. Time-dependent absorp-
tion of therapeutic drugs by the gel of the Greiner Vacuette blood

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