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CASE REPORT

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A Case of Fatal Intoxication from Metformin*

ABSTRACT: A case of fatal intoxication from metformin is presented. The decedent was an obese 58-year-old-woman with type II diabetes, in whom severe lactic acidosis secondary to metformin accumulation was precipitated by acute renal failure. She had been on metformin 500 mg twice a day. Postmortem blood was deproteinated with acetonitrile, washed with dichloromethane, and the resulting supernatant injected into high-performance liquid chromatography system. Separation was performed on a analytical 125 × 4 mm i.d. RP-8 column. The wavelength was set at 235 nm. The mobile phase was acetonitrile (40%), sodium lauryl sulfate, and sodium dihydrogen phosphate adjusted to pH 5.1 (60%) at a flow rate of 1.0 mL/min. The concentration of metformin in postmortem blood was 77.3 μg/mL. The qualitative result was also confirmed by LC/APCI/MS/MS analysis.

KEYWORDS: forensic science, fatal intoxication, metformin, high-performance liquid chromatography analysis, LC/APCI/MS/MS analysis

Metformin (1,1-dimethyl biguanide) is antihyperglycemic, not hypoglycemic. It causes neither insulin release from the pancreas nor hypoglycemia, even when taken in large doses. It has no significant effects on the secretion of glucagon, cortisol, growth hormone, or somatostatin. The main causes of reduced glucose levels during metformin therapy appear to be the increased action of insulin in peripheral tissues and reduced hepatic glucose output due to inhibition of gluconeogenesis. Metformin may also decrease plasma glucose by reducing the absorption of glucose from intestine, but this action has not been shown to have clinical importance.

Patients with renal impairment should not receive metformin. Liver disease, past history of lactic acidosis (of any cause) cardiac failure, or chronic hypoxic lung disease also are contraindications to the use of metformin. All these conditions predispose to increased lactate production and hence to the fatal complications of lactic acidosis. The reported incidence of lactic acidosis during metformin treatment is lower than 0.1 cases per 1000 patient-years, and the mortality risk is even lower (1).

Case History

An obese woman, suffering from many pathologies—type II diabetes treated with insulin and oral hypoglycemic agents (Metbay® 500 Bayer, containing 500 mg of metformin hydrochloride with starch and talc as inactive ingredients), obstructive arteriopathy of the lower limbs, arterial hypertension, previous myocardial infarct, and grade II esophagitis—was taken to hospital due to the onset, about 4 days previously, of vomiting and fever, associated with dyspnea with retrosternal pain, which were partially reduced after administration of nitroderivates (Carvasin® s.l.). After ECG (loss of R wave voltage in V1-V3) and laboratory tests (leukocytes 12.43 10^3μL; hemoglobin 14.6 g/dL; urea 1.43 g/L; creatinine 9.0 mg/dL; glucose 2.76 g/L; LDH 513 U/L; Na 126 meq/L; K 7.4 meq/L; troponin 0.09 ng/mL; lactic acid 103 mg/dL), clinicians diagnosed acute renal failure. The patient was hospitalized in the Nephrology Department where, in view of her metabolic picture, urgent hemodialysis was carried out and interrupted after 2 h. A following arterial hemogasanalysis showed: pH of 7.013, K 6.00 meq/L and HCO_3^- 7.10 mmol/L; after only 45 min these values changed into: pH 7.013, K 6.09 meq/L, HCO_3^- 5.2 mmol/L. The patient was considered to be in critical condition. Another ECG was effectuated and showed alterations attributed to a probable intermittent complete left bundle-branch block; the hematological test results releaved a high value of troponin (2.44 ng/dL).

About 17 h after hospitalization, in spite of treatment received, she died of "cardiogenic shock with lactic acidosis, in a patient suffering from diabetes and postinfarct ischemic cardiopathy." Autopsy and histological findings revealed that the death was due to terminal cardiocirculatory arrest resulting from cardiogenic shock after severe acidosis.

Materials and Methods

Reagents and Chemicals

All reagents were of analytical grade. Metformin was obtained from Sigma–Aldrich (St. Louis, MO). Acetonitrile, dichloromethane, sodium lauryl sulfate, and sodium dihydrogen phosphate were obtained from Merck (Darmastadt, Germany). Ultrapure water (18.2 MΩ) used to prepare the mobile phase was obtained by double distillation and further purified with a Milli-Q system (Millipore, Bedford, MA).

Instrumentation

Chromatographic experiments were performed on an HPLC system consisting of an HP 1050 liquid chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a UV detector connected to a

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HPLC ChemStation. Separation was performed on a analytical 125 × 4 mm i.d. Lichrosphere RP-8 (5 μm particle size) column coupled with a RP-18 guard column (5 μm particle size) (Merck). The mobile phase was 40% solution A and 60% solution B was pumped at a flow-rate of 1 mL/min (107–109 bar). Solution A was acetonitrile; solution B was prepared weighing 1.73 g of sodium lauryl sulfate and 0.828 g of sodium dihydrogen phosphate in 600 mL of water, adjusted to pH 5.1. This was then filtered through a 0.45-μm Millipore filter and degassed ultrasonically before used. The detector wavelength was set at 235 nm and peak areas were measured. In this condition, metformin retention time was 2.81 min at room temperature.

Standard Samples

Standard solution of metformin was prepared in mobile phase at concentration of 75.9 μg/mL. Three standard samples were prepared by adding 200 μL of metformin standard solution to 800 μL of drug-free blood to give a final concentration 15.2 μg/mL. The samples were extracted according to the described method. The drug-free blood is constituted by 1 mL of blood from a subject who had not died due to intake of drugs. Every sample was injected three times (CV% = 3.4).

Extraction of Postmortem Blood Sample

One milliliter of acetonitrile was added to 1 mL of postmortem cardiac blood from the patient. After vortex mixing, the tube was centrifuged at 3956 g for 15 min, and the transferred supernatant was washed with dichloromethane by vortex-mixing for 30 sec. After centrifugation, the aqueous layer was diluted 1:5 with mobile phase and 10 μL of this solution injected into the HPLC/UV instrument.

Results

Because the case history indicated intoxication due to drugs not routinely included in laboratory testing, the extraction method described by Zarghi et al. and Cheng and Chou (2,3) was applied, duly modified, to search for metformin in postmortem blood. Systematic toxicological analysis (STA) was carried out on the sample, to reveal the possible presence of illegal drugs, medicinal drugs, alcohol, volatile substance and/or other poisons, but none was found.

Identification of metformin was based on the retention time (t_R) and on the HPLC/UV analysis carried out on the postmortem blood sample. The qualitative result was also confirmed by LC/APCI/MS/MS analysis (Figs. 1 and 2) in the chromatographic conditions shown in Table 1. Quantitative analysis was carried out in HPLC/UV and the resulting concentration of metformin in the postmortem blood was 77.3 μg/mL.

Discussion and Conclusions

Type II diabetes mellitus—insulin resistance—is the most frequent form of diabetes in Italy. Although not depending on the supplies of exogenous insulin for their survival, patients with this pathology may use insulin in order to correct hyperglycemia. Most patients with type II diabetes are overweight: obesity is in

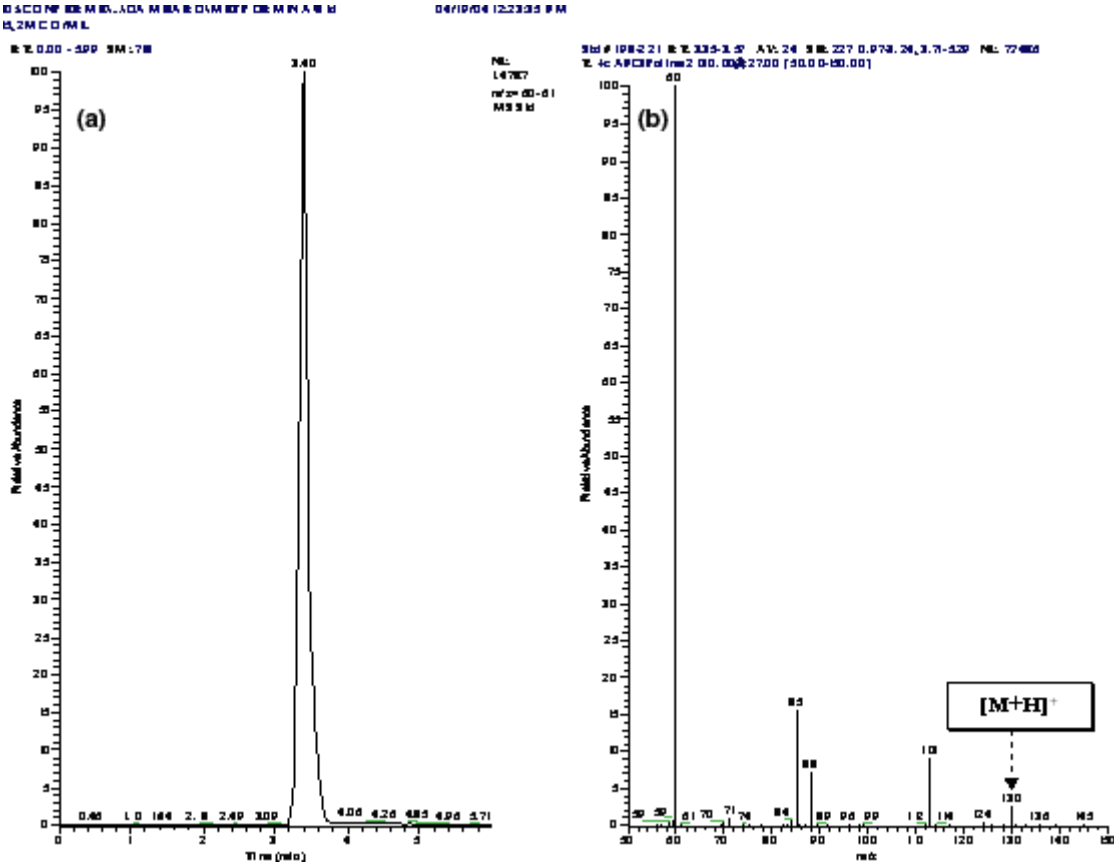

FIG. 1—*(a) LC/APCI/MS/MS chromatogram (m/z 50–150) of 1 mL extract of blank human blood spiked with 15.2 μg of metformin (t_R = 3.40 min) and (b) mass spectra of metformin.*

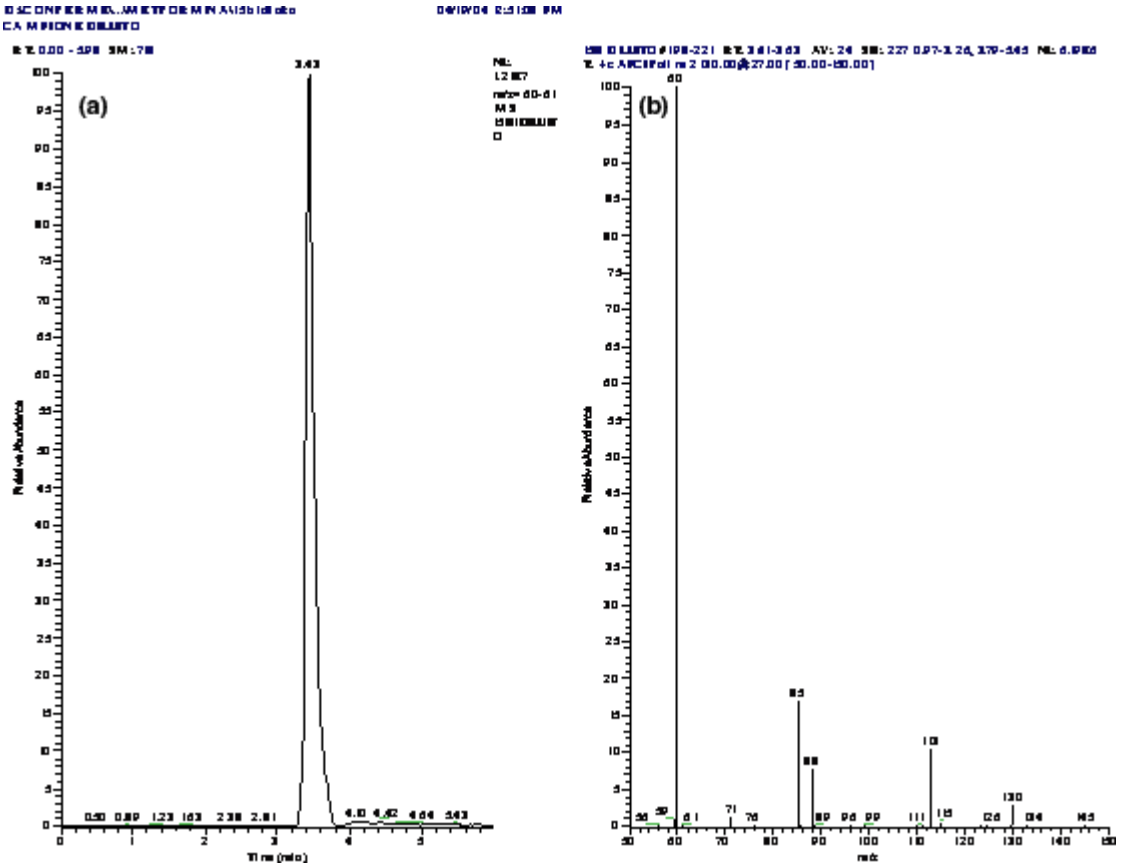

FIG. 2—*(a) LC/APCI/MS/MS chromatogram (m/z 50–150) of postmortem blood sample and (b) mass spectra of metformin.*

TABLE 1—*LC/APCI/MS/MS chromatographic conditions.*

Instrument	Finnigan LCQ^{TM}DUO, equipped with APCI source
Pump	Binary LC Pump 250 (Perkin Elmer)
Column	Luna—CN 150 × 4.6 mm I.D., 3 μm (Phenomenex)
Mobile Phase	30% Ammonium acetate 0.01 M
	70% Acetonitrile–methanol (1–1)
Flow	0.8 mL/min
Source voltage	5.0 kV
Discharge current	4 μA
Capillary temperature	150°C
Vaporizer temperature	450°C
Collision energy	For the fragmentation of molecular ion 130 m/z $[M + H]^+$ at 27% of maximum value

itself a cause of insulin resistance. Although several metabolic complications may sometimes occur, e.g., hyperomolarity or ketoacidosis, in patients with type II diabetes hyperglycemia does not generally involve an immediate risk of death. However, chronic hyperglycemia may lead to micro-angiopathic (retinopathy, kidney disease) and macro-angiopathic complication (myocardial infarct, obliterating arteriopathy of lower limbs) which, as in the case reported here, may considerably influence both the length and the quality of life of patients. Metformin, in the form of 500- and 850-mg tablets, is available in Italy. It is a member of the biguanide family, the drugs which reduce glycemia in patients with type II diabetes without stimulating insulin secretion. Biguanides enhance the exploitation of glucose, both in the intestine and in the skeletal muscle. Whereas in muscular tissue, aerobic glycolysis prevails with complete oxidation of glucose and production of carbon dioxide, and in the intestine anaerobic glycolysis amply prevails with the production of lactate. From the intestine, lactate is transported by means of portal circulation to the liver, where it is normally used for gluconeogenesis. Instead, when the portal concentrations of lactate are very high, liver clearance may be insufficient, causing increased quantities of lactate in the systemic circulation. Lactic acidosis, which initially presents with aspecific symptoms (gastro-enteric disturbances, muscle pain, cramps, asthenia torpor) is a potentially serious event with high mortality. As metformin is mainly excreted through the kidney, it may accumulate in cases of renal insufficiency. In our patient, as there was no information on what therapy she had been following in the days before her hospitalization, and data on renal and hepatic indexes of functionality were not known, it was impossible to establish whether the last dose of metformin was to be reduced or even completely avoided, in order not to increase the risk of lactic acidosis, with consequent involvement of kidney functions. Therefore, it was impossible to establish whether the toxic state of the patient, with high lactacidemia values was pre-existent, or whether it had been triggered by her last dose of metformin. It could only be stated that the metformin values found in blood were comparable with levels reported in the literature (4–7) in cases of lactic acidosis due to accumulation of lactate as a consequence of severe renal failure.

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