Monitoring Drug Concentrations in a Case of Combined Overdosage with Primidone and Methsuximide

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We describe a case of fatal overdose with primidone and methsuximide. During the early phase of the patient’s hospital course we found concentrations of methsuximide, N-desmethylmethsuximide, and primidone in serum that far exceeded the usual therapeutic concentrations, as determined by gas–liquid chromatography. Determination of N-desmethylmethsuximide in peritoneal fluid demonstrated concentrations comparable to those in serum. This led to the therapeutic decision to manage the patient by dialysis. Subsequently, serum samples collected during the course of hospitalization were analyzed quantitatively by gas–liquid chromatography for methsuximide, N-desmethylmethsuximide, primidone, phenobarbital, and diphenylhydantoin. Selected serum specimens were also analyzed by gas chromatography–mass spectrometry, and N-methyl-2-hydroxyethyl-2-phenylsuccinimide, a metabolite of methsuximide not previously described in human serum, was identified by analysis of its mass spectrum.

Additional Keyphrases: gas chromatography • mass spectrometry • new metabolite, N-methyl-2-hydroxyethyl-2-phenylsuccinimide • single-ion chromatography • toxicology

Monitoring of therapeutic agents in serum is widely utilized by neurologists to ensure safe and effective treatment with various anticonvulsant drugs (1, 2). Several of these drugs are metabolized to compounds that also possess anticonvulsant activity or produce symptoms of central nervous system toxicity. In such circumstances, both the parent drug and its pharmacologically active metabolite(s) should be quantitated. Primidone and methsuximide, commonly used to manage various types of seizures, are converted to active metabolites in vivo. Recent studies in man indicate that methsuximide is demethylated to N-desmethylmethsuximide, and the plasma concentration of this metabolite greatly exceeds that of the parent compound in patients treated chronically with methsuximide (3). Primidone is converted in part to phenobarbital, which possesses profound pharmacologic activity, and to phenylethylmalonamide, for which the pharmacologic activity in man is not yet established (4).

Cases of acute overdose with primidone (5, 6) and with methsuximide (7) have been reported and each drug, under this circumstance, produces profound central nervous system depression. Combined massive, fatal overdose with these two drugs, such as occurred in the patient reported here, has not been described previously. We measured serum concentrations of these drugs and metabolites during the course of her hospitalization, which included management with both peritoneal and hemodialysis. In addition, the availability of sequential specimens from this unique case provided us with an opportunity to investigate further the metabolism of methsuximide and primidone, which we did by gas chromatography–mass spectrometry.

Case History

A 29-year-old unconscious white woman was admitted to the Emergency Room of The Johns Hopkins Hospital on March 28, 1975. She had a 10-year history of seizures and during the eight years before admission had been treated with diphenylhydantoin, phenobarbital, and primidone. In August 1973, her neurological status was re-evaluated because of occasional absence seizures, and methsuximide was added to the therapeutic regimen. She was also noted to have an abnormal glucose-tolerance test and was treated by dietary restriction in addition to tolbutamide.

During the month before admission, the patient became progressively more depressed and required
treatment with amitriptyline. She remained depressed, however, and was seen regularly by a psychiatrist. On the evening prior to admission the patient complained of general malaise and had a syncopal episode. Although she awoke later in the evening, she had difficulty walking and acted as if she were intoxicated; she then returned to bed and became unresponsive. Twelve hours later she could not be aroused and was brought to the Emergency Room.

Vital signs on admission included blood pressure, 95/72 mmHg; pulse, 90/min; respiration, 20/min; and temperature, 37.2 °C. The physical examination was unremarkable except for the neurological evaluation, which disclosed an unconscious patient with pupil diameter 7 mm, equal and nonreactive to light; corneal reflexes absent; no sensory response to deep pain; flaccid extremities; and no deep-tendon reflexes.

Laboratory studies demonstrated extremely high serum concentrations of methsuximide (98.5 mg/liter) and primidone (62 mg/liter), and she was admitted to the Medical Intensive-Care Unit.

The patient's hospital course was characterized by an initial respiratory arrest with ventricular tachycardia and cyanosis, but she was quickly resuscitated. Subsequently, she developed hypotension with urine output decreased to 3 ml/h and a systolic blood pressure of 50–60 mmHg. She was first treated with norepinephrine and thereafter she was maintained on dopamine. Because of her hypotension, peritoneal dialysis was begun; however, when her blood pressure stabilized, a shunt was created between her right femoral artery and saphenous vein, and she subsequently underwent two periods of hemodialysis. Her course was characterized by persistent bleeding from the shunt, which necessitated massive transfusion therapy. On the third hospital day, her pupils reacted to light for the first time, and a slight cold caloric reflex was noted. The next day neurological examination showed spontaneous extra-ocular movements and positive corneal reflexes; however, the patient remained flaccid without spontaneous movements. Her level of consciousness did not improve and on the fifth hospital day she had two cardiac arrests and marked hypotension recurred that required pressor therapy. Thereafter her course was further complicated by Gram-negative sepsis and disseminated intravascular coagulation, and she expired on the eleventh hospital day.

**Materials and Methods**

**Comparison Materials**

Diphenylhydantoin and phenobarbital (Sigma Chemical Co., St. Louis, Mo. 63178).

Primidone and phenylethylmalonamide (Ayerst Laboratories, Montreal 101, P.Q. Canada).


p-Toluenesulfonamide (Aldrich Chem. Co., Milwaukee, Wis. 53233)

N,2-Dimethyl-2-(4-hydroxyphenyl) succinimide, referred to hereafter as p-hydroxyphenylmethsuximide, and 2-methyl-2-(4-hydroxyphenyl)-succinimide were kindly provided by Kenneth H. Dudley, Ph.D., of the Center for Research in Pharmacology and Toxicology, School of Medicine, University of North Carolina, Chapel Hill, N. C. 27514.

Methsuximide and N-desmethytmethsuximide were obtained from Parke-Davis & Co., Ann Arbor, Mich. 48106). Standard solutions (100 mg/liter) of each of these compounds were prepared by dissolving 10 mg in 100 ml of methanol.

An internal standard solution containing ethotoin (Abbott Laboratories, N. Chicago, Ill. 60064) and phensuximide (Parke-Davis & Co.) was prepared by dissolving 7.5 mg of each of these compounds in 1 liter of ethyl acetate.

**Extraction Procedure**

Serum (200 μl) was combined in a 16 × 100 mm culture tube (Teflon-lined screw cap) with 50 μl of 2 mol/liter HCl and 5 ml of the internal standard solution. The tubes were extracted for 10 min in an Eberbach shaker at 250 oscillations/min and centrifuged at 2000 rpm for 5 min. The organic phase was transferred to “Concentratubes” (Laboratory Research Co., Los Angeles, Calif. 90036) and concentrated under nitrogen in a water bath at 40 °C. When 5–10 μl of solvent remained in the Concentratube tip the tubes were removed from the evaporator and 50 μl of ethyl acetate was added to each. The tubes were vortex mixed, and 1 μl was injected into the gas chromatograph.

Standard curves were prepared by analyzing samples of normal human serum containing known amounts of methsuximide and N-desmethytmethsuximide. These serum standards were prepared by evaporating to dry-
ness under nitrogen appropriate amounts of the methanolic solutions of each compound and adding 200 µl of drug-free serum. Although phensuximide and ethotoin were equally suitable as internal standards, we used phensuximide to establish peak-height ratios for quantitation of methsuximide and N-desmethylmethsuximide.

Instrument and Instrumental Conditions for Gas Chromatography

We used a Model 5711A gas chromatograph equipped with dual flame-ionization detectors and a Model 7123A 1 mV recorder (both from Hewlett-Packard, Avondale, Pa. 19311). Columns were 122-cm glass, 2 mm i.d., configuration 5 for on-column injection (Hewlett-Packard), packed with 3% OV-17 on 100/120 Gas Chrom Q (Applied Science Labs., Inc., State College, Pa. 16801). The injector temperature was 250 °C, the detector was 300 °C and the oven temperature was programmed 150–270 °C at 16 °C/min.

Gas-Chromatographic Method for Other Anticonvulsant Determinations

Analyses of diphenylhydantoin, phenobarbital and primidone were performed by on-column methylation according to the method of Kananen et al. (8).

Gas Chromatography–Mass Spectrometric–Computer Analysis

For electron ionization mass spectra, we used a Model 5784A gas chromatograph equipped with a heated on-column injection port, interfaced through a membrane separator to a Model 5982A GC/MS system with a dodecapole analyzer, and coupled to a 5933A data system (all from Hewlett-Packard). The gas-chromatographic column was glass, 360 cm × 2 mm i.d., packed with 0.25% neopentyl glycol succinate plus 0.025% phosphoric acid on Chromosorb G-HP, 100/120 mesh (Johns-Manville, Denver, Colo. 80217) (9). The injector temperature was 250 °C; the oven temperature was held at 140 °C for 4 min and then programmed at 8 °C/min to 230 °C and held; and the transfer-line temperature was 250 °C. Mass spectra were recorded at 70 eV ionizing voltage, 200 µA emission current, and at an ion-source temperature of 190 °C. Chemical ionization spectra were obtained by using the same instrument in the chemical ionization mode with the following conditions: no separator was used, methane was used as both carrier and ionizing reagent gas, and spectra were recorded at an ionizing voltage of 300 eV and an emission current of 200 µA.

Results and Discussion

Controls and standards. Figure 1 depicts representative chromatograms of serum specimens extracted according to our procedure. Chromatogram A, with the internal standards phensuximide and ethotoin included, was obtained from serum of a normal individual not receiving anticonvulsants. Chromatogram B was obtained from serum of a normal individual, to which was added 10 µg of methsuximide and 40 µg of N-desmethylmethsuximide per 200 µl. The compounds of interest and the internal standards are well separated from each other and no significant interferences have been observed in serum from patients not on anticonvulsant therapy.

Figure 2 illustrates typical standard curves for methsuximide prepared by the peak-height ratio technique, with phensuximide and ethotoin as internal standards. Linearity was observed between the ratio of

Fig. 2. Standard curves for methsuximide, with use of both ethotoin and phensuximide as internal standards

Fig. 3. Standard curves for N-desmethylmethsuximide, with use of both ethotoin and phensuximide as internal standards
peak height of drug to each internal standard and the amount of drug extracted from serum over the range of 0–15 μg methsuximide.

Figure 3 illustrates typical standard curves for N-desmethylmethsuximide prepared by the peak-height ratio technique, with phensuximide and ethotoxin as internal standards. A linear relation was observed between the ratio of peak height of N-desmethylmethsuximide to each internal standard and the amount of N-desmethylmethsuximide extracted from serum over the range of 0–60 μg of this metabolite of methsuximide.

Data for the patient. A representative chromatogram of serum from the patient, obtained at the time of admission, is presented in Figure 4. The concentrations of methsuximide and N-desmethylmethsuximide in this specimen were 98.5 and 125 mg/liter, respectively. An unidentified peak was observed midway between the chromatographic peaks of phensuximide and N-desmethylmethsuximide. This peak was present in all of the patient’s serum samples, and decreased with time, which suggests that it was also related to the drugs that she ingested.

Serum concentrations of methsuximide, N-desmethylmethsuximide, primidone, phenobarbital, and diphenylhydantoin were monitored throughout the course of hospitalization (Figure 5). At the time of admission, the serum concentrations of methsuximide and N-desmethylmethsuximide were 98.5 and 125 mg/liter, respectively. Thereafter, the serum concentrations of methsuximide declined progressively with time. In contrast, the concentration of N-desmethylmethsuximide in serum increased during the first 36 h of observation to a maximum of almost 300 mg/liter and then declined gradually. The serum concentration of primidone increased from 62 mg/liter at the time of admission to a maximum concentration of 130 mg/liter at 60 h and then decreased with time. The initial concentration of phenobarbital in the patient’s serum was 20 mg/liter and the peak concentration (55 mg/liter), like that of primidone, was observed at 60 h. The concentration of diphenylhydantoin in serum increased from 5.2 mg/liter at the time of admission to a peak of 7.5 mg/liter at 84 h, then decreased progressively.

The initial pattern of high concurrent serum concentrations of methsuximide and its metabolite, N-desmethylmethsuximide seen in Figure 5 has been observed previously only in another case of massive overdosage with methsuximide (7). In contrast, patients treated chronically with therapeutic doses of this drug had plasma concentrations of the N-desmethylmetabolite 700-fold that of the parent compound (3). Strong et al. (3) have suggested a tentative therapeutic range for N-desmethylmethsuximide of 10 to 40 mg/liter, and observed ataxia and lethargy at concentrations exceeding 40 mg/liter. Possible explanations for the high initial serum concentrations of both methsuximide and N-desmethylmethsuximide observed in our patient as well as in the patient reported by Karch (7) included
saturation of the enzyme responsible for demethylation of the parent compound or inhibition of this enzyme by the demethylated product.

At the time of admission, the patient’s serum concentration of primidone was 62 mg/liter, six-fold the upper limit of the therapeutic range for this drug (2). Thereafter the primidone concentration increased to a maximum of 130 mg/liter at 60 h. This time course is most compatible with continued absorption of the drug from the gut. The presence of phenobarbital in our patient’s serum could be due either to formation of this compound in vivo from primidone, or to ingestion of the barbiturate directly: The time course of appearance of phenobarbital in her serum (Figure 5) is compatible with continued absorption of its metabolic precursor, primidone, as well as with sustained absorption of the barbiturate itself.

Because of the high plasma concentrations of methsuximide, primidone, and their active metabolites, it was not possible to determine the contribution of the individual compounds to the profound coma seen in this patient.

After acute overdosage with the drugs this patient took, they would be cleared from the body only by metabolism. Under such conditions in the patient reported by Brillman et al. (5), the plasma half-life of phenobarbital observed in primidone overdosage was 4.5 days, as compared to 15 h for the parent compound. In our patient the concentrations of phenobarbital and primidone declined to about half their peak values by 60 h and 30 h, respectively. We have not calculated half-lives for the clearance of these drugs from serum because of concurrent removal of the compounds by peritoneal and hemodialysis (Figure 5). Peritoneal fluid obtained before the 60-h peritoneal dialysis was begun contained 225 mg of N-desmethylmethsuximide per liter, while serum obtained concurrently contained 278 mg of this drug per liter, which indicates that N-desmethylmethsuximide can cross the peritoneal membrane. This observation prompted the clinical staff to institute and sustain peritoneal dialysis. During the first 2 h of peritoneal dialysis, 280 mg of N-desmethylmethsuximide was removed. When the patient’s blood pressure stabilized, she was also treated by hemodialysis. During a 2.5-h period of the first hemodialysis, approximately 1 g of N-desmethylmethsuximide accumulated in the dialyzer bath. As shown in Figure 5, after 216 h of treatment all drugs in her serum had decreased to either subtherapeutic or nonmeasurable concentrations.

Further information on some components. We thought that the unidentified peak observed in the routine gas-chromatographic analysis of the serum sample (Figure 4) might be another metabolite of methsuximide and therefore we conducted a more detailed investigation by use of combined gas chromatography--mass spectrometry (GC/MS). Three samples of serum were chosen for analysis by GC/MS: one obtained at the time of admission, a second 24 h after admission, and the third at 36 h. Ethyl acetate extracts of these sera were injected on column without derivatization. During the elution of the components from the column, the mass spectrometer was scanned approximately every 2 s through a 40–400 range of m/e and the data were stored on the computer for subsequent analysis. The mass intensities were summed for each spectrum and then normalized to produce a reconstructed chromatogram (Figure 6) analogous to the response from a flame-ionization detector on a gas chromatograph.

The three extracted serum samples analyzed in this manner showed similar peaks in their total-abundance chromatograms; however, the peak heights differed considerably because they represented metabolites of the ingested drugs.

The presence of metabolites of methsuximide other than N-desmethylmethsuximide was investigated in the 36-h sample. Of the 14 peaks present in the chromatogram of this sample (Figure 6), peaks A and C were identified as methsuximide and N-desmethylmethsuximide, respectively, by plotting single-ion chromatograms of the two molecular ions, m/e 203 and 189. The corresponding mass spectra were consistent with literature spectra (3, 10) and spectra of the authentic compounds.

The technique of single-ion chromatography (11) was used to search the GC/MS data for the reported hydroxymethsuximide metabolites (12, 13). Underivatized monohydroxymetabolites of methsuximide exhibit a prominent molecular ion at m/e 219. Figure 7 shows the peaks (B and H) that represent ions of m/e 219. Ex-
amination of the total mass spectra of these peaks indicate that both compounds have a molecular weight of 219.

An ethyl acetate solution of p-hydroxyphenylmethsuximide, one of the known methsuximide metabolites, was injected into the GC/MS and its retention time and mass spectrum were compared to those for components B and H. Component H was thus determined to be p-hydroxyphenylmethsuximide. If component B was also a metabolite of methsuximide, one of three structures was probable: N-methyl-2-hydroxymethyl-2-phenylsuccinimide, N,2-dimethyl-2-phenyl-3-hydroxysuccinimide, or N-hydroxymethyl-2-methyl-2-phenylsuccinimide. The first two of these compounds have previously been reported as urinary metabolites of methsuximide in the rat, guinea pig, and human (13). The N-hydroxymethyl compound was ruled out by the intense m/e 134, which is attributable to loss of the -CONCH$_3$CO- moiety similar to methsuximide itself. A tentative identification of component B as N-methyl-2-hydroxymethyl-2-phenylsuccinimide is based on the intense m/e 132 ion, which corresponds to the molecular ion of 2-phenyl propenal formed by the loss of hydrogen from the 134 ion.

Because of the large amount of N-desmethylmethsuximide present in the patient’s serum, we also searched for its corresponding p-hydroxymetabolite. However, there was no peak present that had a retention time or mass spectrum corresponding to that of authentic 2-methyl-2-(4-hydroxyphenyl)-succinimide.

Several components could not be readily identified because their electron ionization spectra did not exhibit molecular ions. For this reason, we obtained a chemical-ionization (methane) GC/MS analysis from the 36-h sample. The chemical ionization spectra of components...
L and F clearly showed their molecular weights to be consistent with those of primidone and phenylethylmalonamide, respectively. Chemical ionization spectra of N-desmethylmethsuximide, p-hydroxymethylmethsuximide, and the hydroxylated metabolite of the drug tentatively identified as N-methyl-2-hydroxyethylmethyl-2-phenyl-succinimide, are shown in Figure 8. Because the process of chemical ionization involves a low energy transfer of a proton, the mass spectra show very little fragmentation, with the largest peak usually being the protonated molecular ion (MH+), peaks appearing at M+29 (M+C3H5) and M+41 (M+C3H7) are also common when methane is used as the reagent gas (14).

Component D (Figure 6) was compared to authentic tolbutamide and to p-toluene sulfonamide, the thermal decomposition product of tolbutamide. The retention time and mass spectra of component D were identical to those of authentic p-toluene sulfonamide. Moreover, the chemical ionization mass spectrum of component D showed its major peak at m/e 172 consistent with the protonated molecular ion of p-toluenesulfonamide. Matin and Knight (15) reported that N-1-methyl tolbutamide, a closely related compound, decomposed in part to N-1-methyl-p-toluenesulfonamide both in the injector port and ion source of their GC/MS system.

Component E was identified as tri-2-butoxyethylphosphate, a plasticizer, from B-D Vacutainer stoppers, by comparison of its electron ionization mass spectrum to the published spectrum of this contaminant (16). Component J was identified, from its mass spectrum, as cholesterol. Component G appears to have a molecular weight of 228. The base peak of its electron ionization spectrum is 130; however, this material has not been identified. The very minor components K and M appear to be steroidal type compounds. Diphenylhydantoin was detected in trace amounts by plotting the single-ion chromatogram of the molecular ion.

We thank the house staff of The Johns Hopkins Hospital for their diligence in collecting and identifying the large number of clinical samples obtained from this patient during her hospitalization and for providing us with a summary of her clinical course.

References