Comparison of Glutethimide Concentration in the Serum and Cerebrospinal Fluid of Humans in Drug Overdose

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Glutethimide concentrations were measured gas chromatographically in the serum and cerebrospinal fluid (CSF) of patients in coma caused by overdose of this drug. Piperidine was used as an internal standard. In the first serum samples collected from each patient, the glutethimide concentration ranged from 0.9 to 3.1 mg/dl. No patient had a CSF glutethimide concentration of more than 0.8 mg/dl. If one excludes patients who regained consciousness in less than 7 h, the seven remaining cases may be described by the following equation: \( y = 16.06 x^{1.06} \) where \( y \) = duration of coma (in h) and \( x \) = maximum serum glutethimide concentration (in mg/dl). The correlation coefficient for this equation was 0.911.

Additional Keyphrase: gas chromatography

Glutethimide overdoses can lead to serious states of central nervous system depression that can, if not adequately treated, terminate in death. Knowledge of the concentration of the drug in serum is useful clinically, if serum drug concentrations and the degree of depression are related. There is general agreement in the literature that this correlation for glutethimide concentration is only a poor one (1, 2).

Because cerebrospinal fluid (CSF) bathes the central nervous system it is reasonable to speculate that glutethimide concentrations in nervous tissue are more accurately reflected by use of this fluid as a sample rather than serum. We find no published data on glutethimide concentrations in human CSF after an overdose. We studied a series of patients in coma due to glutethimide, by sampling their blood stream and lumbar CSF, and report the results here.

Materials and Methods

Venous blood samples were allowed to clot and the serum was removed after centrifugation, the elapsed time being about 45 min. CSF was removed by lumbar puncture. The next series of operations were carried out in a 40-ml ground-glass-stoppered centrifuge tube. A Hamilton syringe was used to add 10 µl of the internal standard, piperidine solution (10 mg/10 ml of reagent grade methanol), to 1.0 ml of sample; 1.0 ml of sodium acetate buffer (pH 4.8, 1.0 mol/liter) and 10 ml of chloroform (J. T. Baker Chemical Co., Phillipsburg, N. J. 08865; listed as

“GC-spectrophotometric grade”) were then added. The glutethimide was extracted by agitating the mixture on a vortex-type mixer for 30 s. The centrifuge tube was centrifuged briefly to separate the phases, and 5 ml of the nonaqueous (lower) layer removed to a 12-ml conical centrifuge tube. The chloroform was evaporated in a water bath at 50–60 °C, under a gentle stream of nitrogen, 25 µl of chloroform was used to dissolve the residue and wash down the walls of the tube, and 4 µl of the solution was injected into a gas chromatograph with a Hamilton 10-µl syringe.

A Model 990 gas chromatograph was used (Perkin-Elmer Corp., Norwalk, Conn. 06856) with a stainless steel column 185 cm long and 4 mm in diameter. The column was washed with dichlorodimethylsilane 5 ml/100 ml chloroform. The column was then packed with “Chromsorb Q” (80–100 mesh) coated with “3% OV-17” (Supelco, Inc., Bellefonte, Pa. 16823). A carrier flow of 30 ml/min (30 lb./in.2) of nitrogen was maintained by flow controllers. Air pressure was supplied at 30 lb./in.2 and hydrogen, for the flame ionization detector, was maintained at 30 lb./in.2 and supplied by an Elhygen unit (Milton Roy Co., St. Petersburg, Fla. 33733). Transparent cylinders, filled with moisture-indicating adsorbent and molecular sieve 5 A (Supelco, Inc., Bellefonte, Pa. 16823), were placed in the air and nitrogen gas lines to remove moisture and contamination. The range knob was set at ×10 and the attenuation knob at ×16, equivalent to 160th of the maximum signal. The analog signal from the chromatograph was connected to a Honeywell recorder, 1 mV full scale, and the chart paper speed was 5 min/in. The injector block temperature was 300 °C, as was the detector compartment. Initial oven temperature was 180 °C. After a time lag of 6 min, it was rapidly programmed to 190 °C (rate: 24 °C/min) and held at this final temperature. This temperature schedule was a slight variation of Barrett’s (3) procedure for sedative screening. The retention time of glutethimide was determined by comparison with a pure standard. Glutethimide and hexobarbital are not resolved by this system. In order to distinguish these two compounds, an unknown sample is injected simultaneously with trimethylanilinium hydroxide to convert them to their methylated derivatives in the injector block of the chromatograph. The methylated glutethimide and hexobarbital have different reten- tion times and can thus be separated.

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A standard curve was constructed by adding known concentrations of glutethimide to serum not containing this drug. The peak height ratio (glutethimide/piperidione) and peak area ratio were plotted against the added glutethimide concentrations. A linear relationship was found to exist, at least up to 10 mg/dl serum. Reproducibility was better with the peak-height ratio than the peak-area ratio technique. The areas were measured by peak height times the width at half-height. All the results presented in this paper were obtained by the former measurement method.

**Results**

The gas-chromatographic analysis of a patient’s serum containing glutethimide usually produces a variety of peaks. Only one is the unmetabolized glutethimide. The other peaks are metabolites or are associated with glutethimide ingestion. They are not due to pyrolysis in the injector block or decomposition on the column, as glutethimide showed only one peak when extracted after addition to control sera and injected under similar conditions. As many as seven peaks could be observed.

Sunshine et al. (4) found similar results with chloroform extraction and proposed washing the extract with sodium hydroxide to remove most of the non-glutethimide compounds. They found that petroleum ether had the same effect as sodium hydroxide, i.e., essentially glutethimide was extracted. Ambre and Fischer (5) used ethyl ether as an extraction solvent and reported finding seven substances. Both groups found two of these peaks in control sera, so it would appear that the least four peaks in addition to glutethimide are noted in humans. We have seen up to seven peaks also, but control sera have not demonstrated any corresponding peaks.

The metabolite patterns observed by gas chromatography of serum extracts can be quite varied. Figure 1 shows a chromatogram with mostly glutethimide present. Figure 2 shows a compound that appears to be the major metabolite formed, eluting just after glutethimide. Figure 3 illustrates the formation of two additional peaks. These three peaks related to glutethimide are quantitatively the largest seen on the chromatogram. Of course, it is possible that very polar compounds would not be extracted by chloroform and would go undetected. It is possible to have all combinations of relative peak heights, depending on when the sample was removed in relation to the time of ingestion. The longer the time since ingestion, the less glutethimide will be found in the serum compared to the associated peaks, the actual pattern depending on the patient.

The CSF patterns of glutethimide concentration tend to follow those found in the serum. However, the relative peak heights are different.

Serum glutethimide levels are graphed against time in Figure 4. The initial values, at zero time, were the first samples drawn from each patient. This
method of presentation was chosen as the time elapsed since drug ingestion was usually not known. Three of nine patients showed concentrations in serum that were still increasing, starting at 1.6, 2.1, and 3.1 mg/dl and rising to a maximum of 2.1, 2.9, and 3.3 mg/dl, respectively. The lowest value seen with a patient in coma, by our definition (see below), was 0.9 mg/dl. The person with the greatest serum glutethimide concentration showed no pain or light responses when first seen and did not awake until he had been in the hospital for more than three days. After the first maximum concentration was reached in all patients, the concentrations determined later never were observed to rise again at lower concentrations. This implies that there was no discernible recycling from depot sites or from enterohepatic reabsorption, just a constant equilibrium between gradually decreasing release from storage with a probable first-order rate of disposal.

Cerebrospinal fluid glutethimide concentrations are plotted vs. time in Figure 5. Here also, zero time indicates the time of collection of the initial blood sample and not the withdrawal time of the CSF sample, as some of the CSF samples were removed after the first blood specimens and not simultaneously. Since the same scale is used for the serum and CSF the two plots may be compared directly. Only two CSF specimens were available for each of six patients. None of the cases showed an elevation after the first CSF sample and no CSF value was greater than 0.8 mg/dl. Three patients had a concentration in CSF of 0.8 mg/dl and one patient was found to have a concentration of 0.7 mg/dl. The three patients whose glutethimide concentration was 0.8 mg/dl had concentrations in serum of 1.5, 2.1, and 3.1 mg/dl. After 24 h the concentrations in the CSF of three of six patients were found to be 0.30 mg/dl or greater, and after 48 h only one patient’s glutethimide concentration had not dropped below 0.30 mg/dl. This was the patient (P.P.) with the highest serum concentration in the present series. His initial CSF concentration of 0.8 mg/dl had only dropped to 0.65 mg/dl after 58.5 h. Unfortunately, no more CSF specimens were available and he did not wake up until 82.5 h after the first blood sample. It is obvious that his case is unique, as his arousal is so prolonged compared to the other patients. One case (V.D.) had been on L-dopa therapy for severe parkinsonism and this might have had some effect on the results found for the CSF.

Discussion

Possibly the rate of decrease in serum glutethimide was related to the maximal concentration attained in serum. Accordingly, a plot was made of the maximum serum concentration against the rate of decrease found with the succeeding sample for each person. The same procedure was carried out with the CSF specimens. The results are shown in Figure 6. There does seem to be a definite relationship. All of the results fall reasonably close to a linear correlation except for one patient whose renal clearance was the greatest of those studied. This was in a 26-year-old girl who had been reported to have had an earlier drug overdose episode.

The CSF values were never above 0.8 mg/dl, and the lowest serum maximum attained was 0.9 mg/dl, so there is no overlapping of results on Figure 6. It is interesting to note that the rates of change for CSF are similar to what they would be expected to be in serum if there were any serum concentrations corresponding to those in the CSF.
Table 1. Maximum Glutethimide Concentration vs. the Concentration at Time of Waking (Eight Subjects)

<table>
<thead>
<tr>
<th>Serum</th>
<th>Cerebrospinal fluid</th>
<th>Duration of coma, h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum</td>
<td>Waking</td>
</tr>
<tr>
<td>2.7</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>3.3</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
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<td>1.8</td>
<td>0.8</td>
</tr>
<tr>
<td>2.1</td>
<td>2.0b</td>
<td>0.4</td>
</tr>
<tr>
<td>2.9</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>2.3</td>
<td>1.3</td>
<td>0.4</td>
</tr>
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</table>

a Estimated by extrapolation, see text.
b Receiving L-dihydroxyphenylalanine for severe parkinsonism.

What is the lowest concentration in serum at which the CSF will exhibit a value of 0.8 mg/dl? This seems to be in a serum concentration range of 1.0–1.5 mg/dl. Up to 1.0 mg/dl the CSF value was approximately 44% of the serum glutethimide concentration. Why does the CSF glutethimide concentration never exceed 0.8 mg/dl? As Davson (6) discusses, there is active transport of materials across the choroid plexus as well as diffusion over much of the surface comprising the blood–brain barrier. Apparently the rate of transport and diffusion of glutethimide from all sites is not rapid enough to prevent the inflow of cerebrospinal fluid from diluting the CSF glutethimide to a value lower than serum. Compounds can also be taken up by the brain from the CSF, so this is another possible mechanism for creating a lower CSF concentration. Our sampling site was the lumbar region, and it has been found for various materials that their concentration in cervical CSF can be higher than in the lumbar CSF (6). This might further account for a lower CSF concentration than that found in serum.

Clinical records were examined to elucidate the point at which the patients awoke from coma. Many were observed to partially awake and oscillate into and out of consciousness, a common finding with glutethimide (1). In our study, the time when the patient’s eyes opened spontaneously and he responded verbally was chosen as the waking state.

Table 1 lists the waking glutethimide levels in serum and CSF as well as the maximum serum concentration noted for eight patients along with the duration of coma. One case (M.C.) is not included because no serum sample was taken near the time when the patient woke up. One CSF value (P.P.) was estimated by extrapolating the one in Figure 6 to the waking time. The serum values at the time of arousal ranged from 0.4 to 1.8 mg/dl. The corresponding CSF values on waking ranged from 0.15 to 0.58 mg/dl. The lowest waking serum value (0.4 mg/dl) was noted with serum maxima of 2.3 and 2.9 mg/dl, while the highest serum maximum (3.3 mg/dl) was associated with a waking serum value of 0.7 mg/dl. Both serum and CSF waking glutethimide concentrations exhibited wide ranges and no concentration could be selected as the value a patient must have to awaken.

If the patients waking sooner than 7 h are excluded, the seven remaining cases may be described by the following equation, which relates the duration of coma to the maximum serum glutethimide concentration observed: \( y = 16.06 x^{0.06} \) where \( x \) is the maximum serum glutethimide level and \( y \) is the duration of coma, in hours. The correlation coefficient was 0.911.

The literature is replete with conflicting results (1, 2, 8) by the method of Goldbaum et al. (7). Sunshine et al. (4) state that this includes glutethimide metabolites, and so studies carried out with this technique are suspect due to nonspecificity. Ambre and Fischer (5) used gas chromatography on plasma specimens. They report four cases were in coma at 0.5 to 1.2 mg/dl and another woke up at 1.2 mg/dl. Sunshine et al. (4) also used gas chromatography on blood samples. They found all but one patient in coma on admission. One person awoke at a glutethimide concentration of 1.8 mg/dl and the others at 1.0 mg/dl or less, with a coma duration of 32–61 h after admission.

Methodological differences along with the difficulty in assessing the neurological state probably contribute substantially to the disagreement among authors as to correlations with serum glutethimide concentrations.

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References