

CASE REPORT

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The Analysis of Metronidazole in Human Serum: An Unusual Overdose Case

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ABSTRACT: A case of attempted suicide involving metronidazole in conjunction with ethanol. The chemical and toxicological findings will be discussed.

KEYWORDS: toxicology, metronidazole, suicide, high performance liquid chromatography, antibiotics, overdose, Flagyl®

Suicide attempts through the ingestion of readily available drugs are a common phenomenon. In this case, however, the use of an antibiotic was unusual. In this paper we report the ingestion of an extremely large quantity of metronidazole 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole in conjunction with ethanol. Only a few other cases of accidental or suicidal overdoses have been reported. These include a case where a pregnant woman injected 4.2 g of metronidazole [1]. Single oral doses of up to 15 g have also been reported [2].

Case History

A 27-year-old female was brought to the Emergency Department by paramedics at about 2 p.m. Her main complaint was severe vomiting which she stated had been present since morning.

From the history, it was determined that she had recently filled a prescription for 80 tablets of Flagyl® (metronidazole) [3] (G.D. Searle & Co., Chicago IL) of which she had only taken 2 before her injection of the remainder of the supply. Therefore, it was established that she had ingested 78 tablets (19.5 g) of Flagyl. In addition, her history revealed that she had swallowed the tablets during the prior evening with approximately 500 cm³ (1 pt) of vodka. Her admitting laboratory studies including electrolytes, blood gases, kidney function (for example, blood urea nitrogen and creatinine), and liver enzymes were normal. She had a

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blood alcohol level of 0.06% (w/v). She was admitted to the Intensive Care Unit for observation where she was treated supportively. Two days post admission she was transferred to a regular medicine ward and watched for two more days before discharge. Throughout her hospital stay her major symptom remained nausea—which finally disappeared on her last hospital day. As this represents the highest recorded overdose, we undertook a study to determine what her blood levels of metronidazole were.

Two blood samples were taken from the patient for the purposes of determining the metronidazole level. The first was obtained at approximately 2:30 p.m. the day of admission (some 16 h after injection) and the other was obtained at approximately 11:30 a.m. the next day (some 36 h after injection).

Experimental Procedure

This procedure is a modification of that of Kosobud and Chen.³ Methylene chloride, ethanol, and methanol distilled in glass (Burdick and Jackson) were used without further purification. The methanol:water solution (30:70 v/v) used as the eluting solvent in the high performance liquid chromatograph (HPLC) was degassed using a Millipore filtering apparatus using a 0.45- μ m filter. Carbonate buffer, pH 9.8, 0.1M was prepared from reagent grade sodium carbonate and sodium bicarbonate. Metronidazole and SC23190 (internal standard) were supplied by Searle. A Waters HPLC equipped with a Model 6000 solvent delivery system and a Model 450 variable wavelength absorbance detector was used for analysis. The effluent was monitored at 313 nm. The eluting column was a Waters model RC 100 radial compression system, reverse phase, micro Bondapak C₁₈ column. The eluting solvent was methanol:water (30:70 v/v). The flow rate was 2.0 mL per minute. The recorder was a dual pen Omniscribe[®] (Houston Instruments).

Preparation of Standards for Linearity and Recovery Studies

A stock solution of metronidazole (1 mg/mL) in ethanol was prepared as was a similar stock solution of SC23190. Eight standards were prepared from the stock solutions of metronidazole and SC23190 for the standard curve. The concentrations of the standards were 2, 5, 10, 20, 40, 60, 80, and 100 μ g/mL in metronidazole and 10 μ g/mL in SC23190.

Solutions of the patient's serum for the recovery studies were prepared at the 10- and 100- μ g/mL levels and extracted. These were compared to ethanolic solutions at the same concentrations.

Extraction Procedure

One-millilitre of the serum standard or sample of the patient's serum was transferred to a 30-mL glass tube with a ground glass stopper. One-millilitre of the carbonate buffer was then added to each sample. Then 25 mL of methylene chloride was added to each. The tubes were then shaken for 20 min on a mechanical shaker. The two phases were separated by centrifuging at 3000 rpm for 5 min. The aqueous layer was removed by vacuum aspiration and the organic layer was transferred to a glass vial and evaporated to dryness at 35°C under a stream of nitrogen. The residue was dissolved in 200 μ L of mobile phase. This solution was then injected into the HPLC.

³L. Kosobud and P. Chen, "Simultaneous Determination of Serum and Urine Levels of Metronidazole and Hydroxymethylmetronidazole by High Pressure Liquid Chromatography." private communication, 1981.

Results

Both samples of the patient's blood were centrifuged to remove red cells and then frozen until analyzed. For analysis, the serum samples were diluted as follows: for the sample withdrawn the first day, 0.5 mL of serum was diluted with 12.5 mL of saline. For the other sample, 0.5 mL of serum was diluted with 10 mL of saline. For each determination 1 mL of the diluted samples was used. The SC23190 was then added to achieve a concentration of 10 $\mu\text{g}/\text{mL}$.

Figure 1 is the standard curve constructed from the data obtained from the eight standards prepared. The curve is obtained by plotting the ratio of the peak height response of the drug to that of the internal standard versus the concentration of the drug in micrograms/millilitres. The concentrations of the metronidazole in the patient's serum samples were determined by calculating the peak height response ratios and interpolating the standard curve to obtain the concentrations which were then corrected for dilutions.

Figure 2 is a sample chromatogram for metronidazole and SC23190 taken from the patient samples and one of the standards. Under these instrumental conditions, the metronidazole has a retention volume of 7.8 mL and the SC23190 has a retention volume of 9.3 mL.

Using peak height ratios and interpolation of the standard curve, the patient sample withdrawn at admission had an average value of 640 $\mu\text{g}/\text{mL}$ and the sample taken the next day had an average value of 240 $\mu\text{g}/\text{mL}$.

Recovery studies using the same extraction methods gave an average value of 80.5% recovery of metronidazole.

Discussion

The HPLC method for this analysis works very well with a minimum of difficulty in extraction. Other methods for the analysis of metronidazole have been reported in the literature. Quantitative analysis by spectrophotometry is described by Ings et al [4]. Durel et al [5] describe a method for the rapid detection and determination of metronidazole in urine.

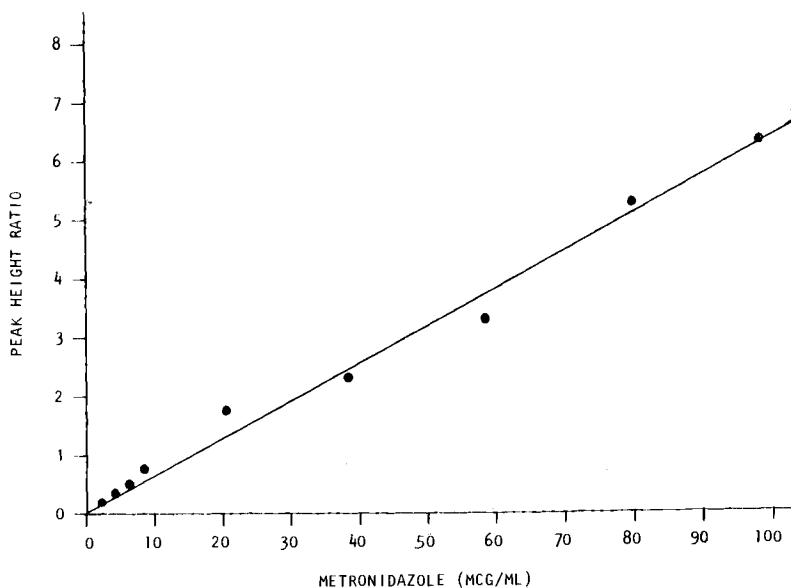


FIG. 1—Assay linearity.

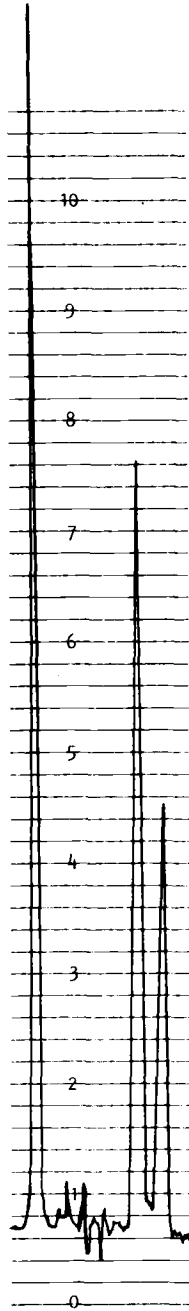


FIG. 2—HPLC of metronidazole and SC23190.

The reported average half-life for therapeutic doses of metronidazole in healthy humans is about 8 h [2]. Since the first serum level of 640 $\mu\text{g}/\text{mL}$ was obtained approximately 16 h after injection of the tablets, the maximum serum concentration would have been in the range of about 2500 $\mu\text{g}/\text{mL}$ since the drug reaches peak plasma concentration after only 1 to 2 h [2]. This means that the drug exhibited a low toxicity since the patient complained only of nausea throughout the episode. Several neurotoxic effects have been reported with lower doses than found in this case [2].

Some 20 h later the serum level had dropped from 640 to 240 $\mu\text{g}/\text{mL}$. This is not consistent with a half-life of 8 h, but because the dosage levels were so high in this case and because of the additional injection of alcohol, such data as half-life and peak plasma concentration may not apply.

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