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Mass Fragmentographic Determination of Plasma Etomidate Concentrations

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Abstract □ The intravenous anesthetic etomidate was measured in human plasma by mass fragmentography. The method is accurate, sensitive, and specific. Results of the analyses indicate that after a single 0.3-mg/kg iv dose of etomidate, there are at least three phases in its disappearance from human plasma. Detectable plasma concentrations exist for more than 6 hr after injection.

Keyphrases □ Etomidate—mass fragmentographic analysis, human plasma □ Mass fragmentography—analysis, etomidate in human plasma □ Hypnotics—etomidate, mass fragmentographic analysis, human plasma

Etomidate is a new imidazole hypnotic used intravenously for induction of anesthesia. Compared to thiopental, the standard intravenous anesthetic, etomidate produces less cardiovascular and respiratory depression (1, 2). Like thiopental, etomidate induces sleep rapidly (10–15 sec) and has a short duration of action (2). Unlike the short-acting barbiturates, however, it does not produce a "hangover" effect (3) due to its rapid disappearance from nervous tissue and plasma and the dependence of this process upon rapid metabolism rather than redistribution (4). However, etomidate disposition has not been studied extensively.

Because etomidate has promise as an anesthetic induction agent (5), a sensitive, specific assay for use in future clinical pharmacological studies was needed. The developed method is based on combined GLC–mass spectrometry and single ion monitoring, a technique called mass fragmentography.

EXPERIMENTAL

Reference Compounds—Authentic etomidate sulfate [(*R*)-(+)-ethyl 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate sulfate] and propoxate hydrochloride [(+)-propyl 1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate hydrochloride] were used as received[1]. Aqueous solutions of propoxate, 1 and 2 μg/ml, were used as internal standards. All etomidate concentrations are expressed in terms of free base.

GLC–Mass Spectrometry—A quadrupole gas chromatograph was interfaced to a mass spectrometer[2] with a glass jet separator and glass transfer lines. A 1.5-m (5-ft) × 2-mm i.d. glass column was silanized and packed with 5% OV-225 on 100–120-mesh Supelcoport[3]. Helium was the carrier gas at a flow rate of 20 ml/min. The temperature of the injection port was 250°; the column, separator, and transfer line temperatures were 230, 260, and 230°, respectively. Operating conditions for the mass spectrometer were: electron energy, 70 ev; emission ion current, 200 amp; and power supply, 2.5 kv.

Complete mass spectra of authentic etomidate and propoxate were first obtained by using the solid probe inlet to the mass spectrometer. The identities of these compounds as they eluted from the chromatograph were confirmed by comparison of their complete mass spectra to those of the authentic compounds.

These operating conditions also were used for mass fragmentography. The intensity of the *m/e* 105 and 77 ions was monitored using a programmable multiple-ion monitor[4] (at a sensitivity of 10^{-9}) and a dual-pen strip-chart recorder. The *m/e* 105 ion was monitored for the detection and quantitation of both etomidate and propoxate since it was the most abundant. The mass marker was used to center the ion window with a window width of less than 1 amu. The mass marker was not in operation during sample analyses.

Procedure—Blood samples were obtained from patients who underwent anesthetic induction with etomidate for eye or ear surgery. All patients were otherwise healthy, had normal liver and kidney function, and were taking no other medication. Each received 10 mg of diazepam and 0.3 mg of scopolamine intramuscularly 30 min prior to etomidate injection.

An indwelling cannula was placed in an antecubital fossa vein, and a 10-ml control blood sample was withdrawn. Etomidate (0.3 mg/kg iv) was then administered over 30 sec into an antecubital fossa vein of the other arm. After induction with etomidate, anesthesia was maintained with nitrous oxide–oxygen and enflurane.

Blood samples of 10 ml were withdrawn through the cannula into heparinized glass syringes. Samples were taken at 1, 2, 4, 8, 15, and 30 min after injection and then hourly to 6 hr. The blood samples were immediately transferred to 15-ml glass-stoppered centrifuge tubes containing heparin and 10 μl of saturated potassium fluoride solution (to inhibit esterase activity) (6). The plasma was separated by centrifugation at 2000 rpm for 15 min and then frozen and stored at −15° until extracted.

Because of the wide range of etomidate concentrations, the 0–30-min samples were processed separately from later samples. Standard plasma samples containing etomidate concentrations ranging from 1.25 to 0.10 μg/ml were extracted with the 0–30-min patient plasma samples. Aliquots, 3 ml, of 0.05 *M* phosphate buffer, pH 7.4, and 1.0 ml of 1.0-μg/ml propoxate hydrochloride internal standard solution were first added to a 1.0-ml aliquot of each plasma sample. These samples were then subjected to the extraction procedure described below.

Patient plasma samples from later sampling times (1–6 hr) were analyzed with a separate set of standard plasma samples containing 0.25–0.001 μg of etomidate/ml. Aliquots, 1 ml, of a 0.2-μg/ml propoxate hydrochloride internal standard solution were added to 4.0-ml aliquots of these samples. These samples were then subjected to the following extraction procedure, which is a modification of that suggested by Wynants *et al.* (7):

[1] Courtesy of Janssen Pharmaceutica, Beerse, Belgium.
[2] Finnegan model 3200E.
[3] Supelco, Inc., Supelco Park, Bellefonte, Pa.
[4] Finnigan model 1015-80 PROMIM.

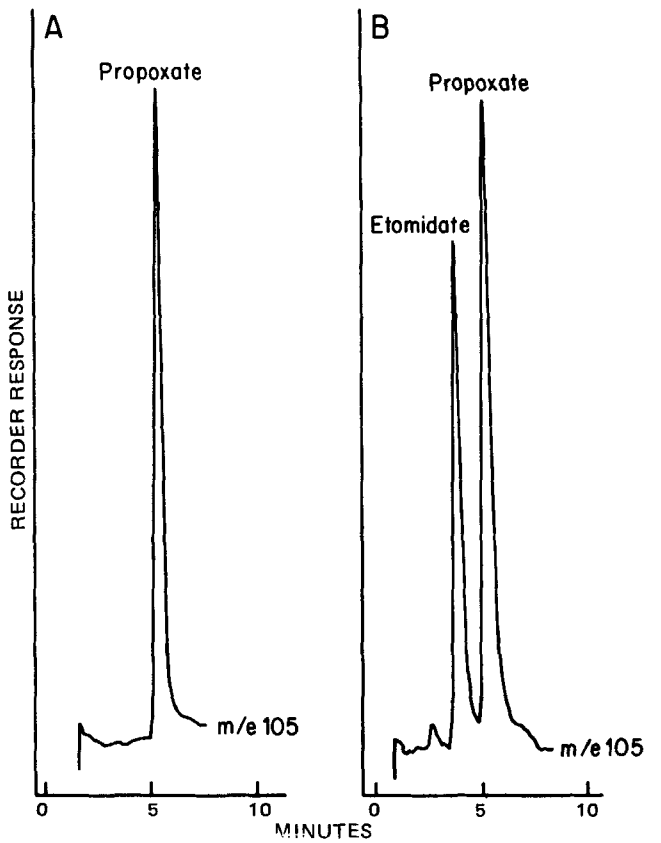

Figure 1—*Mass fragmentograms of plasma extracts from Patient 2, indicating elution position of etomidate and the internal standard propoxate. Key: A, control sample; and B, 3-hr sample containing 21.2 ng of etomidate/ml.*

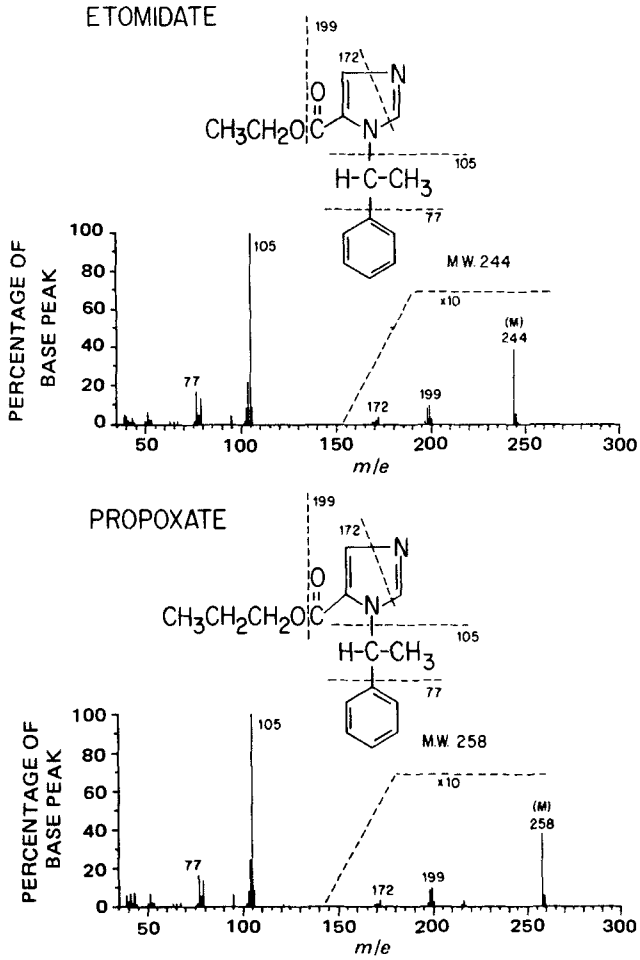

Figure 2—*Mass spectra of authentic etomidate and propoxate.*

1. Add 1.0 ml of 0.05 *M* borate buffer, pH 10, and mix on a vortex mixer.
2. Add 5.0 ml of ether, shake on a horizontal shaker for 10 min, centrifuge at 1500 rpm for 5 min, and quick freeze the plasma layer in a dry ice–acetone bath. Transfer as much of the organic phase as possible to another 15-ml screw-capped centrifuge tube and seal with a polytef-lined cap. (The quick-freeze step facilitates the recovery of the organic phase.)
3. Thaw frozen plasma samples in a 40° water bath and repeat Step 2. Centrifuge at 2000 rpm for 10 min, quick freeze, and combine the ether extracts.
4. Add 5.0 ml of 0.5 *M* H_2SO_4 to ether extracts, shake for 10 min, centrifuge at 1500 rpm for 5 min, and remove all ether by aspiration.
5. Add 3.0 ml of ether to the acidic aqueous extract; shake, centrifuge, and aspirate as in Step 4.
6. Add 1.0 ml of 3.0 *M* NH_4OH and mix on a vortex mixer.
7. Add 3.0 ml of methylene chloride; shake and centrifuge as in Step 4. Remove as much of the aqueous layer as possible by aspiration.
8. Carefully transfer the methylene chloride extract to an evaporating tube.
9. Evaporate to dryness.
10. Take up the residue in 10–50 μl of *n*-hexane and inject 1–5 μl on the chromatograph column.

A typical chromatogram is shown in Fig. 1. The magnitude of the detector response indicates the intensity of the *m/e* 105 ion.

Each standard curve was constructed by plotting the ratio of etomidate to propoxate peak heights against etomidate concentration. Standard curves were linear over the entire range of concentrations measured.

RESULTS AND DISCUSSION

Recovery of etomidate from extracted plasma was determined by comparing absolute peak height ratios of extracted standards to those obtained from unextracted primary standards. The actual recovery determined was 72 ± 3%.

The coefficient of variation obtained by extraction and analysis of five replicate standards at 100, 50, 10, 5, and 1 ng/ml of plasma was 2.4%. Reproducibility did not vary significantly from day to day.

As little as 100 pg of etomidate could be detected by injection on the chromatograph column. The practical limit of assay sensitivity is 1 ng/ml of plasma, which is at least 10-fold more sensitive than the method of Wynants *et al.* (7). The specificity of the mass fragmentography technique allows extraction of large volumes of plasma without interference from endogenous substances.

The conventional mass spectra of etomidate and propoxate are shown in Fig. 2. The base peak for each compound is at *m/e* 105. The proposed structures of the major fragments also are indicated. The *m/e* 105 ion is probably stabilized as the methyl tropylium structure (8). The *m/e* 199 ion arises from loss of the alkoxy side chain (9), while the *m/e* 172 ion may result from ring cleavage and loss of hydrogen cyanide, a pattern characteristic of imidazoles (10). Other possible origins for the *m/e* 199 and 172 ions are unlikely, since these ions have identical abundance in the mass spectra of both propoxate and etomidate. The *m/e* 77 ion probably represents the phenyl ring fragment.

During mass fragmentographic analysis, the *m/e* 77 ion also was monitored. The ratio of its intensity to that of the *m/e* 105 ion served as a check against the possibility that an endogenous component of plasma with the same retention time was contributing to the size of the *m/e* 105 peak (11).

Since the major metabolite of etomidate in humans is the corresponding carboxylic acid, it has been assumed that plasma pseudocholinesterase may hydrolyze the drug (6), although apparently this has not been studied. A 10-μl aliquot of a saturated solution of potassium fluoride was added to all blood samples to inhibit esterase activity. Etomidate concentrations in samples treated in this manner were unchanged for at least 2 hr at room temperature and for at least 1 month when frozen and stored at −15°.

Concentrations of etomidate in the plasma of patients given 0.3 mg/kg iv for induction of anesthesia are shown in Fig. 3. All values are the result of duplicate determinations. The large variation in concentrations in the 1–4-min samples probably reflects incomplete mixing of the drug within

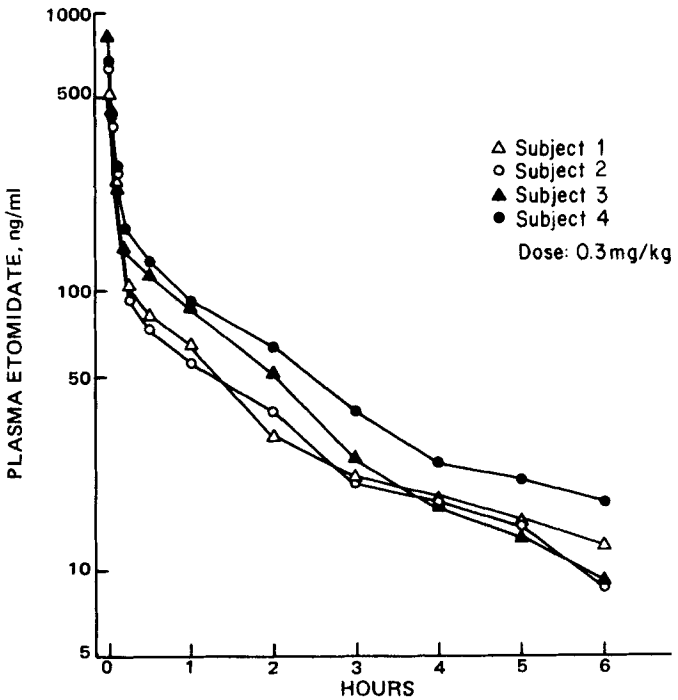

Figure 3—*Plasma concentrations following 0.3 mg of etomidate sulfate/kg iv. Concentrations in the 1-min samples from Patients 1 and 4 were 1170 and 1200 ng/ml, respectively.*

the vascular volume. Plasma etomidate concentrations in these patients at 6 hr after the injection were still well above the limits of assay sensitivity. Therefore, etomidate is detectable in plasma for several hours longer than previously indicated (7).

The semilogarithmic plot of these plasma level data (Fig. 3) indicates that there are at least three phases in the plasma disappearance of etomidate. This assay provides the sensitivity necessary to measure terminal phase plasma concentrations important in drug kinetic studies (12). A pharmacokinetic study involving a larger number of patients is currently in progress.

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In Vitro Adsorption of Doxorubicin Hydrochloride on Insoluble Calcium Phosphate

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Abstract □ The adsorption of doxorubicin hydrochloride, a potent antitumor agent, on solid tribasic calcium phosphate was studied *in vitro*. A Langmuir adsorption isotherm at pH 7.4 and the maximum adsorption capacity of tribasic calcium phosphate were established. Tribasic calcium phosphate was chosen as a model for solid bone samples, which are stained with doxorubicin in patients who have received long-term doxorubicin therapy.

Keyphrases □ Doxorubicin hydrochloride—adsorption on solid tribasic calcium phosphate *in vitro* □ Adsorption—doxorubicin hydrochloride on solid tribasic calcium phosphate *in vitro* □ Calcium phosphate, tribasic—adsorption of doxorubicin hydrochloride *in vitro*

Doxorubicin (I), an anthracycline antibiotic, has demonstrated antitumor activity against various solid tumors (1) as well as certain hematologic malignancies (2). Some serious complications encountered in the use of I are related to bone marrow depression, cardiac toxicity, stomatitis, and alopecia (3). Perhaps the most significant is bone marrow depression, primarily of leukocytes, which requires careful monitoring. In patients who have received extended I therapy, autopsies have shown that cross sections of bone tissue are stained with I (2, 4). Since solid bone samples are also stained, the process by which I is deposited in these areas is of interest.

The I molecule contains an anthraquinone moiety. Some mono- and dihydroxyanthraquinones are good chelating agents for several metal ions (5, 6). However, these compounds only form calcium chelates at pH 10–12, much greater than physiological pH. It was originally assumed that I would chelate a calcium ion in solution *in vivo* and be deposited in the outer layers of solid bone. Preliminary studies showed that I does not interact appreciably with calcium ions in solution at pH < 10 even when calcium ions are present in large excess.

To approximate bone samples, solid tribasic calcium