High-Performance Liquid Chromatographic Assay for Etomidate in Human Plasma: Results of Preliminary Clinical Studies Using Etomidate for Hypnosis in Total Intravenous Anesthesia

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Abstract □ A sensitive and specific high-performance liquid chromatographic assay was developed for the measurement of etomidate in human plasma following extraction of the drug and the internal standard. Using 0.5-ml aliquots of plasma, the assay was linear in the concentration range of 20–2000 ng of etomidate/ml of plasma. This method was used to evaluate a preliminary clinical study of an etomidate infusion regimen for hypnosis in a total intravenous anesthesia protocol in 23 patients. The average duration of the infusion was 30 min, and awakening and alertness occurred 20 and 36 min after the termination of the infusion, respectively, at the respective plasma concentrations of 297 and 214 ng/ml. These results and this assay will be used to design and evaluate an improved etomidate infusion regimen.

Key phrases □ Etomidate—high-performance liquid chromatography, human plasma, infusion, hypnosis in total intravenous anesthesia □ Hypnotic agents—etomidate, infusion, hypnosis in total intravenous anesthesia, high-performance liquid chromatography, human plasma □ High-performance liquid chromatography—etomidate, human plasma, infusion, hypnosis in total intravenous anesthesia

Etomidate, a carboxylated imidazole, is an hypnotic agent which rapidly produces sleep after intravenous administration (1) and has only a minimal and transient effect on respiration (2) and no effect on cardiovascular stability (3). Etomidate is primarily metabolized in the liver by ester hydrolysis to the inactive carboxylic acid (4, 5). Recovery of consciousness is rapid due to both redistribution and metabolism of the drug (6). These properties suggest that an etomidate infusion would be suitable for the maintenance of hypnosis in a totally intravenous anesthesia regimen.

Several methods for the measurement of etomidate in plasma have been reported, but these use instrumentation that may not be readily available and require the extraction of relatively large volumes of plasma. The method of Wynants *et al.* (7) uses GC with an alkali flame ionization detector (AFID) and is sensitive to 10 ng/ml but requires the extraction of 3 ml of plasma. Van Hamme *et al.* (8) reported a GC–MS assay that is sensitive to 1 ng/ml using extracts of 1–4 ml of plasma. Sensitivity to 5 ng/ml was achieved by De Boer *et al.* (9) using capillary GC with an nitrogen–phosphorus detector. A GC–AFID method was also developed by Haring *et al.* (10) which requires the extraction of only 1 ml of plasma, but is sensitive to only 30 ng/ml.

It was the purpose of the present study to develop a simple yet sensitive high-performance liquid chromatographic (HPLC) method for measuring plasma etomidate concentrations. This method was then used to measure the plasma etomidate concentrations in blood samples obtained during an infusion of etomidate for hypnosis in a totally intravenous anesthesia regimen and up to 6 hr postinfusion. The resulting plasma concentration *versus* time curves were then used to evaluate the infusion protocol and determine the plasma etomidate concentrations at awakening and alertness under the conditions of the anesthetic.

EXPERIMENTAL

Reagents—Etomidate sulfate [*R*-(+)-ethyl 1-(α-methylbenzyl)imidazole-5-carboxylate sulfate], propoxate hydrochloride [*R*-(+)-propyl 1-(α-methylbenzyl)imidazole-5-carboxylate hydrochloride], *R*-(+)-1-(α-methylbenzyl)imidazole-5-carboxylic acid, mandelic acid, and hippuric acid were used as received[1]. All organic solvents were LC[2] or analytical[3] grade. The sulfuric acid, ammonium hydroxide, sodium hydroxide, and sodium borate were analytical grade[4].

Instrumentation—Plasma etomidate concentrations were measured using a constant-flow HPLC system consisting of a solvent delivery system[5], a universal injector[6], a radial compression separation unit[7] liquid chromatography cartridge and cartridge insert packed with 10-μm CN resin[8], and a fixed-wavelength UV detector fitted with a 254-nm wavelength kit[9]. The chromatograms were recorded, the peaks were identified and integrated, and the concentrations were reported on the basis of the internal standard area ratio method by a reporting integrator[10].

Extraction—Five-milliliter blood samples were obtained by syringe through a 16-gauge polytef catheter, previously inserted in an arm vein in each patient, at appropriate time intervals (described below) and transferred to heparinized blood collection tubes[11]. The plasma samples were removed after centrifugation of the blood for 10 min at 2000 rpm and stored at −30° until extracted in duplicate according to a modification of the procedures of Wynants *et al.* (7) and Van Hamme *et al.* (8).

A 500-μl aliquot of plasma, 50 μl of a 3.423-μg/ml ethanolic solution of propoxate hydrochloride and 100 μl of 0.05 *M* borate buffer (pH 10) were added to a conical centrifuge tube and mixed. This mixture was extracted twice with 3.0 ml of hexane–ether (9:1, v/v) by mixing for 5 min on a slowly rotating mixer. The mixtures were centrifuged for 5 min at 2000 rpm, and the hexane–ether layers were transferred to a conical centrifuge tube containing 3.0 ml of 0.5 *M* H_2SO_4 and mixed for 5 min. The mixture was centrifuged for 5 min at 2000 rpm, and the hexane–ether layer was removed and discarded. The acidic aqueous extract was washed with 3.0 ml of hexane–ether for 5 min; the mixture was centrifuged for 5 min at 2000 rpm and the hexane–ether layer was removed and discarded. The acid extract was made basic by adding 1 ml of 3 *M* NH_4OH. This solution was extracted with 4 ml of methylene chloride, and then centrifuged for 5 min at 2000 rpm. The organic phase was evaporated to dryness under reduced pressure at 30° [12], and the residual material was reconstituted with 100 μl of methanol; 15 to 30 μl was injected into the HPLC. Recovery was evaluated by comparing the etomidate–propoxate area ratios for standards containing 2000, 200, and 20 ng of etomidate

[1] Courtesy of Janssen Pharmaceutica, Beerse, Belgium.
[2] Waters Associates, Milford, Mass.
[3] Mallinckrodt, Inc., Paris, Ky.
[4] Mallinckrodt, Inc. or J. T. Baker, Phillipsburg, N.J.
[5] Waters Associates Model M-45.
[6] Waters Associates Model U6K.
[7] Waters Associates Model RCM-100.
[8] Waters Associates Radial-PAK CN and CN Guard-PAK.
[9] Waters Associates Model 440.
[10] Model 3390A; Hewlett-Packard, Avondale, Pa.
[11] Model A3206KA; Becton, Dickinson and Co., Rutherford, N.J.
[12] Model R-110; Buchi/Brinkmann, Westbury, N.Y.

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(as the base)/ml of plasma in which the internal standard solution was added after the extraction to those in which it was added before the extraction.

Chromatography—The HPLC system was as described above; the mobile phase was methanol–water (54:46) delivered at 1.8 ml/min. The etomidate concentrations, in terms of free base, were determined by the internal standard area ratio method.

The linearity, accuracy, and precision of the assay were assessed by the measurement of the etomidate concentration of replicate plasma standards made by adding known amounts of etomidate from stock solutions to blank human plasma. These plasma standards contained 20, 50, 100, 200, 500, 1000, and 2000 ng/ml of etomidate as the base.

Clinical Study—Twenty-three women scheduled to have minor gynecologic surgery, but otherwise in good health, participated in this institutionally approved study after giving informed consent. The patients were premedicated with morphine sulfate (0.1 mg/kg im) 60–90 min prior to induction of anesthesia. An intravenous infusion of 5% dextrose in Ringer's lactate was started through a 20-gauge polytef catheter into a large vein of the arm opposite that used for blood sampling; drugs were administered intravenously through this tubing at the injection site closest to the catheter. Three to five minutes prior to the induction of anesthesia, fentanyl (100 μg iv) and droperidol (2.5 mg iv) were administered. Anesthesia was induced with 0.3 mg/kg of etomidate (a 2-mg/ml solution in propylene glycol) administered intravenously over 1 min. Maintenance of hypnosis was accomplished with a continuous intravenous infusion of 0.1% etomidate (1 ml of a 125-mg/ml etomidate solution in 10% ethyl alcohol diluted to 125 ml with 0.9% NaCl or 5% dextrose in water) by an infusion pump at a rate 0.1 mg/kg/min for the first 10 min then at 0.01 mg/kg/min for the remainder of the anesthesia. Further analgesia was provided, when necessary, using fentanyl (50 μg iv). Pancuronium was given, when necessary, to facilitate tracheal intubation and for muscle relaxation and was reversed at the end of the operation by pyridostigmine (10 mg iv) and glycopyrrolate (0.4 mg iv). Patients were ventilated with oxygen or oxygen-enriched air.

The times to awakening and alertness were determined as the time from discontinuing the etomidate infusion to the time the patients responded to verbal stimuli and the time the patients were oriented in time and place, respectively. Once they were alert, they were asked to attempt to arise and walk beside their bed every 15 min until they were able to walk unaided for a distance of ~2 m.

Blood samples were obtained 1, 2, 5, 10, 15, 30, and 45 min into the etomidate infusion and 5, 10, 15, 30, 45, 60, 75, 90, 120, 180, 240, and 360 min postinfusion. Plasma etomidate concentrations at awakening and alertness were determined, when necessary, by interpolation of the plasma concentration *versus* time curves for each patient. The elimination half-life was determined by a log-linear regression analysis of the plasma concentration *versus* time relationship during the elimination phase.

RESULTS AND DISCUSSION

Chromatographic Assay—Chromatograms of extracts of the 2000-ng/ml and the 20-ng/ml plasma etomidate standards are illustrated in Fig. 1. The carboxylic acid metabolities of etomidate eluted in <1.2 min, well before the esters etomidate and propoxate, and therefore did not interfere with the assay. None of the drugs administered concomitantly in the present protocol were found to interfere with the assay.

The accuracy and precision of the HPLC technique was evaluated by measuring the concentration of etomidate in spiked plasma standards six times each over the period of a week (Table I). A linear regression analysis of the standard etomidate concentrations from 20 to 2000 ng/ml *versus* etomidate–propoxate area ratios verified the linearity of the extracted standard curve ($r = 0.999$; $y = 267.9x + 3.5$). The average recoveries for four replicate samples at 2000, 200, and 20 ng/ml were 82.4, 82.6, and 82.4%, respectively.

Clinical Study—The average (±*SD*) age and weight of the patients were 35 (±10) years and 64 (±12) kg, respectively. No patient complained of pain on injection and no signs of venous irritation were observed. All patients fell asleep rapidly, remained asleep throughout the operation, and had no recall of operative events. Mild, almost imperceptible, myoclonia occurred at induction in two patients, but lasted less than 15 sec in each case. There were no clinically significant side effects during maintenance, and changes in blood pressure and heart rate occurred only in relation to the administration of other drugs and surgical stimulation. Awakening was complicated in six patients by the desire to turn to the lateral position, muscle twitching, restlessness, or hypertonus in the period between first awakening and complete alertness. Postoperative nausea or vomiting was observed in four patients.

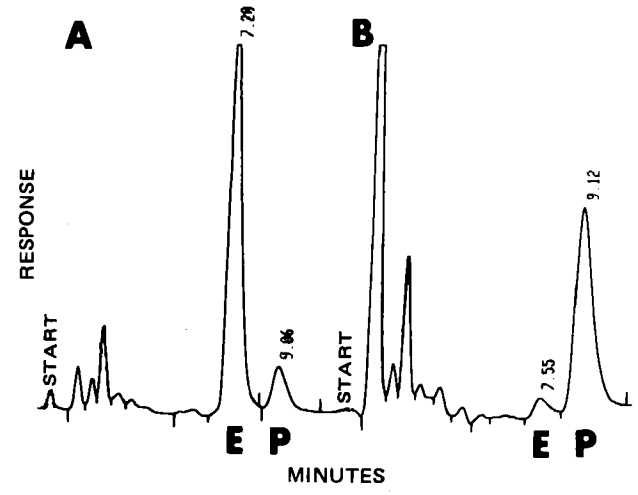

Figure 1—*Chromatograms of etomidate (E) standards extracted from blank plasma with the internal standard propoxate (P). Key: (A) plasma sample containing 2000 ng of etomidate/ml; (B) plasma sample containing 20 ng of etomidate/ml. The retention times for the peaks are as indicated.*

Table I—Accuracy and Precision for the Plasma Etomidate Assay [a]

Etomidate Added, ng/ml	Etomidate Measured, ng/ml [b]	Mean Error, ng/ml	Relative Error, %	*CV*, %
20	23.6 ± 3.2	3.6	18.	13.6
50	53.0 ± 4.1	3.0	6.0	7.7
100	102.5 ± 8.5	2.5	2.5	8.3
200	205.6 ± 6.5	5.6	2.8	3.2
500	494.7 ± 11.1	5.3	1.1	2.2
1000	984.0 ± 36.0	16.0	1.6	3.7
2000	2008.0 ± 51.0	8.0	0.4	2.5

[a] $n = 6$. [b] Mean ± *SD*.

A typical plasma etomidate concentration *versus* time curve during and after the infusion is shown in Fig. 2, and pertinent clinical results for the 23 patients are listed in Table II. The average length of anesthesia in these patients was 30 (±26) min, and they awoke at an average of 20 (±9) min after the end of the etomidate infusion. The mean plasma etomidate concentration on awakening was 297 (±74) ng/ml; Schüttler

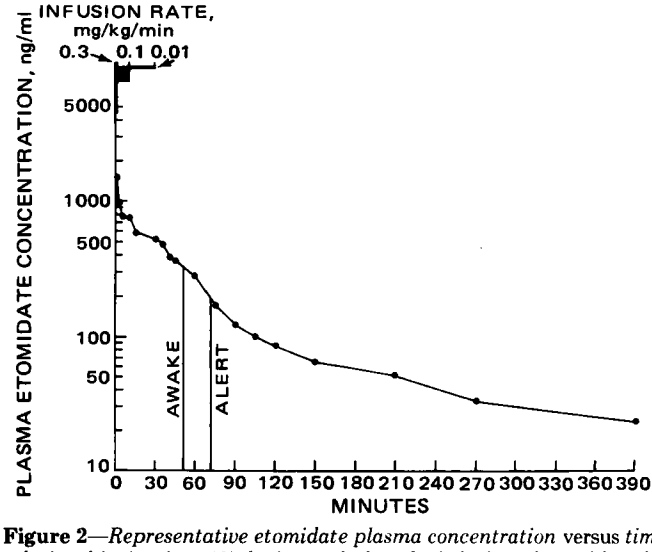

Figure 2—*Representative etomidate plasma concentration* versus *time relationship (patient 10) during and after the infusion of etomidate for hypnosis in total intravenous anesthesia. The rates and duration of the infusion as well as the times of and plasma concentrations at awakening and alertness are indicated.*

Table II—Time and Plasma Etomidate Concentrations at the End of the Infusion, Awakening, and Alertness and Elimination Half-Life of Etomidate

Patient	Age, year	Weight, kg	Infusion		Awakening		Alertness		Elimination Half-Life, hr
			Duration, min	Plasma Conc. at End, ng/ml	Postinfusion Time, min	Plasma Conc., ng/ml	Postinfusion Time, min	Plasma Conc., ng/ml	
1	26	50	46	473	8	280	25	195	1.1
2	27	57	22	650	15	424	40	290	4.2
3	32	54	24	584	28	240	58	155	2.7
4	29	74	18	1178	12	410	42	200	—[a]
5	46	63	22	430	19	310	26	270	3.5
6	32	68	18	897	24	390	39	270	—[a]
7	25	57	23	303	6	210	7	190	—[a]
8	27	56	45	448	15	200	18	170	5.0
9	27	60	18	528	12	310	22	220	4.7
10	29	54	31	525	21	310	41	190	2.6
11	29	70	136	509	34	170	—[a]	—[a]	—[a]
12	44	74	14	2948	22	350	52	170	1.9
13	32	60	45	411	18	220	25	160	6.2
14	32	67	49	282	21	220	51	145	3.3
15	30	50	42	540	16	300	26	270	2.0
16	45	61	18	521	10	338	25	180	—[a]
17	44	85	18	1091	13	370	25	270	1.0
18	41	61	17	625	15	235	30	180	—[a]
19	27	52	22	729	26	375	44	270	2.9
20	28	56	25	1152	33	370	41	310	2.9
21	41	58	10	2885	25	265	36	215	3.4
22	65	90	15	1572	38	220	43	210	3.0
23	46	90	20	1020	39	310	72	170	5.5
Mean (±*SD*)	35 (±10)	64 (±12)	30 (±26)	883 (±718)	20 (±9)	297 (±73)	36 (±15)	214 (±50)	3.3 (±1.5)

[a] —Not determined.

et al. (11), using the analytical method of Wynants *et al.* (7), found the minimal plasma etomidate concentration producing an hypnotic effect to be ~300 ng/ml. The patients were alert an average of 36 (±15) min after the end of the etomidate infusion, and this was associated with an average plasma etomidate concentration of 214 (±50) ng/ml. The patients were able to walk unaided for ~2 m an average of 87 (±29) min following the termination of the infusion. The elimination half-life was 3.3 (±1.5) hr, which is longer than that of Schüttler *et al.* (11) but shorter than that of Van Hamme *et al.* (12). The patients generally remained drowsy for 4–6 hr after leaving the recovery room.

While these results demonstrate that anesthesia can be satisfactorily accomplished with an etomidate infusion and bolus injections of fentanyl, droperidol, and pancuronium, the present protocol is not optimal. An etomidate infusion regimen for hypnosis in totally intravenous anesthesia should be designed to maintain plasma concentrations above the awakening concentration while avoiding dose-related side effects and keeping awakening time under 10 min regardless of the duration of anesthesia. This could theoretically be accomplished using the plasma etomidate data of the present study and the etomidate pharmacokinetic data from the intravenous bolus study of Van Hamme *et al.* (12). The HPLC method reported here could then be easily used to evaluate such a designed infusion regimen.

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