Anesthesiology
50:227–229, 1979

Laboratory Reports

Hydrolysis of Etomidate

M. M. Ghoneim, M.D., and Michael J. Van Hamme, Ph.D.†*

The site of hydrolysis of etomidate, a new intravenous anesthetic, and effects of bis-(p-nitrophenyl) phosphate (BNPP), a specific inhibitor of the hepatic microsomal hydrolase, on the duration of action and elimination of etomidate were investigated. Etomidate concentrations ranging from 0.1 to 1.0 μg/ml were incubated with fresh human and canine plasmas. Half the samples were treated with potassium fluoride solution to inhibit the esterases. Sleep times and the plasma kinetics of etomidate were investigated in two groups of dogs, half of which received etomidate, 1.6 mg/kg alone; the other half were pretreated with BNPP. There was no significant change in the concentration of etomidate after incubation with plasma in the presence or absence of fluoride. Dogs that received etomidate alone (controls) slept 4.8 ± 1.0 (SEM) min after the first injection and 4.6 ± 0.9 min after the second. The difference was not statistically significant. In the study group, mean durations of sleep were 4.6 ± 1.2 min after the first injection and 7.2 ± 0.9 min after BNPP treatment. The difference was significant ($P < 0.01$). Plasma clearances of etomidate were 37 ± 4 l/hour in the control group and 31.9 ± 2.8 l/hour in the pretreated group. The difference was not significant. It is concluded that etomidate is not hydrolyzed in human or canine plasma. The hypnotic action of the drug is prolonged after treatment with BNPP, but the mechanism of this interaction remains to be elucidated. (Key words: Anesthetics, intravenous: etomidate. Enzymes: pseudocholinesterase. Liver: metabolism, microsomes.)

ETOMIDATE, a carboxylated imidazole, has a short duration of action, presumably because of rapid hydrolysis in the plasma.[1] The action of the drug in the presence of an abnormal pseudocholinesterase enzyme and its interaction with drugs such as succinylcholine have been questioned.[2] The drug may also be metabolized in the liver. Previous pharmacokinetic studies in our laboratory[3] have suggested a hepatic extraction ratio of 0.5. Bis-[p-nitrophenyl] phosphate (BNPP) is claimed to be a specific inhibitor of the hepatic microsomal hydrolase,[4] which has no hypnotic activity of its own. The present study was undertaken to determine whether plasma esterases hydrolyze the drug in human and canine species and to elucidate the effects of inhibition of hepatic carboxylic ester hydrolase on sleep time and elimination of etomidate.

* Professor.

† Assistant Research Scientist.

Received from the Department of Anesthesia, University of Iowa, College of Medicine, Iowa City, Iowa 52242. Accepted for publication June 28, 1978.

Reported in part at the annual meeting of the American Society of Anesthesiologists, New Orleans, October 1977.

Address reprint requests to Dr. Ghoneim.

Methods

Samples of fresh heparinized plasma with normal cholinesterase and pseudocholinesterase activities and dibucaine numbers were obtained from two volunteers.‡ A range of etomidate sulfate§ concentrations from 0.1 to 1.0 μg/ml was prepared in plasma. Solutions were incubated in glass flasks in a metabolic shaker at 37 C/80 oscillations/min. The samples of plasma in half the flasks were treated with saturated potassium fluoride solution, 2.5 μl/ml, to inhibit esterase activity.[5] Two-milliliter volumes were taken from the solutions 0, 5, 60 and 240 min, after incubation and were immediately transferred to glass scintillation vials to which aqueous saturated potassium fluoride solution, 10 μl, had been added. The plasma was immediately frozen and stored at −15 C until the drug was extracted.

Samples of plasma were allowed to thaw at room temperature prior to extraction, and a 1.0-ml volume was taken from each. A freshly prepared aqueous solution of propoxate hydrochloride,§ used as internal standard, was diluted with doubly distilled water to produce concentrations similar to those of etomidate in the prepared plasma solutions. A 1.0-ml volume was then added to each plasma sample containing a similar initial etomidate concentration. After addition of 1.0 ml buffer, pH 10, plasma samples were extracted, then analyzed by mass fragmentography.[6] Any hydrolysis of etomidate would be reflected as a decrease in the etomidate/propoxate peak height ratio, which would be anticipated to be close to unity in the absence of hydrolysis. The same procedure was repeated with canine plasma with normal cholinesterase and pseudocholinesterase activities and dibucaine numbers.

Ten mongrel dogs each weighing approximately 15 kg were randomly divided into two groups. A control group received etomidate, 1.6 mg/kg, intravenously on two occasions, with successive adminis-

‡ Institute of Agricultural Medicine, College of Medicine, University of Iowa.

§ Janssen Pharmaceutica, Beerse, Belgium.

0003-3022/79/0300/0227 $00.65 © The American Society of Anesthesiologists, Inc.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/50/3/227/300331/0000542-197903000-00012.pdf by guest on 04 June 2023

TABLE 1. Etomidate Concentrations after Incubation with Human and Canine Plasmas

Plasma	Concentration of Etomidate in Plasma*	Etomidate/Propoxate Peak Height Ratio ± SD	
		Fluoride-treated Plasma†	Untreated Plasma
Human	0.1	1.15 ± 0.01‡	1.06 ± 0.02
	0.2	1.21 ± 0.01	1.19 ± 0.02
	0.5	1.11 ± 0.01	1.21 ± 0.02
	1.0	1.26 ± 0.04	1.30 ± 0.01
Canine	1.0	1.32 ± 0.01§	1.41 ± 0.02

* μg/ml of the base.
† Plasma was treated by addition of 2.5 μl of a saturated aqueous solution of potassium fluoride per ml plasma.
‡ Results are averages ± SD of samples taken at 0 min and 5 min, one hour, and four hours after incubation of etomidate with plasma. Samples were analyzed in duplicate each time.
§ Sampling times were 0 min, 5 min, 30 min, one hour, and two hours after incubation.

trations separated by at least six days. A study group received etomidate the first time, and at the next treatment bis-[*p*-nitrophenyl]phosphate (BNPP)[¶] was administered intravenously in a dose of 50 mg/kg 30 min prior to the etomidate injection.[4] BNPP was prepared as a 1 per cent solution in physiologic saline solution and was injected over a 10-min period. Sleep time was measured by reappearance of the eyelash reflex.

Another ten mongrel dogs were divided into two groups. A control group received etomidate, 0.5 mg/kg, injected into a vein of the forelimb over a 30-sec period. Blood samples were collected in heparinized glass syringes through an indwelling cannula in the jugular vein 4, 8, 15, and 30 min after injection, and then hourly for six hours. Each sample was immediately transferred to a glass-stoppered centrifuge tube containing saturated aqueous potassium fluoride solution, 10 μl, and sodium heparin, 100 units. After centrifugation at 2,000 rpm for 30 min the plasma was separated and stored at −15 C.

The second group was pretreated with 50 mg/kg BNPP administered intravenously over 10 min; 30 min later etomidate was administered and blood samples collected. A 5 per cent solution of dextrose in lactated Ringer's solution was administered to the dogs in both groups to compensate for the volumes of blood removed for analysis.

Etomidate was extracted, then quantified by mass fragmentography. Data were analyzed with the aid of an IBM 360/65 computer, and a program for nonlinear least-squares regression analysis.[7] The student t test was used to evaluate the differences between experimental and control conditions. $P < 0.05$ was considered significant.

¶ Aldrich Chemical Company, Inc., Milwaukee Wisconsin.

Results

There was no significant decrease in the concentration of etomidate after incubation with human or canine plasma in the presence or absence of fluoride treatment when one takes into consideration that the coefficient of variation of the analytic method was 2.4 per cent (table 1).

Dogs that received etomidate alone (controls) slept 4.8 ± 1.0 (SEM) min after the first injection and 4.6 ± 0.9 min after the second. The difference was not significant. In the study group, mean durations of sleep were 4.6 ± 1.2 min after the first injection and 7.2 ± 0.9 min after BNPP pretreatment, a significant increase ($P < 0.01$).

There was no significant difference in the plasma levels of etomidate or in kinetic parameters between the control dogs and those pretreated with BNPP except for k_{12} and k_{21}/k_{12} (table 2). In a three-compartment open pharmacokinetic model[3] k_{12} is the rate constant for drug transfer between the central compartment and a shallow peripheral compartment,

TABLE 2. Kinetics of Etomidate in Control Dogs and in Dogs Pretreated with BNPP*

	Control Mean and Range Values		Pretreated Mean and Range Values	
A_1 (μg/ml)	0.21	(0.27–0.19)	0.31	(0.44–0.24)
A_2 (μg/ml)	0.18	(0.20–0.03)	0.16	(0.20–0.04)
A_3 (μg/ml)	0.04	(0.05–0.03)	0.04	(0.06–0.01)
λ_1 (hr^{-1})	16.60	(19.09–4.15)	19.92	(23.84–4.32)
λ_2 (hr^{-1})	2.98	(3.78–0.36)	2.83	(3.66–0.24)
λ_3 (hr^{-1})	0.35	(0.48–0.24)	0.30	(0.43–0.17)
k_{10} (hr^{-1})	2.41	(2.61–1.89)	2.75	(3.24–1.65)
k_{12} (hr^{-1})	4.83	(5.68–1.83)	7.54	(7.70–2.03)†
k_{21} (hr^{-1})	9.89	(11.89–0.79)	9.47	(13.90–0.68)
k_{13} (hr^{-1})	2.09	(3.42–1.31)	2.56	(3.22–1.98)
k_{31} (hr^{-1})	0.71	(0.94–0.60)	0.73	(0.98–0.29)
$t_{1/2\lambda_1}$ hr	0.04	(0.17–0.04)	0.03	(0.16–0.03)
$t_{1/2\lambda_2}$ hr	0.24	(1.91–0.18)	0.26	(2.93–0.19)
$t_{1/2\lambda_3}$ hr	2.12	(2.88–1.43)	2.63	(4.09–1.62)
λ_n‡/k_{10}	0.15	(0.24–0.09)	0.12	(0.24–0.05)
k_{21}/k_{12}	2.08	(2.37–0.43)	1.25	(1.80–0.23)†
k_{31}/k_{13}	0.41	(0.60–0.18)	0.27	(0.37–0.14)
V_c (l)	17.10	(28.70–12.66)	14.30	(19.46–8.86)
V_d area (l)	102.75	(149.84–66.06)	111.35	(169.33–81.36)
Plasma clearance Cl_p (l/hr)	36.97	(54.28–30.60)	31.86	(43.61–25.91)

* 50 mg/kg, intravenously.
† $P < 0.05$.
‡ n = 2 for a two-compartment model and n = 3 for a three-compartment model.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/50/3/227/300331/0000542-197903000-00012.pdf by guest on 04 June 2023

which usually includes richly perfused organs such as the brain and viscera. k_{21}/k_{12} reflects drug movement "to and fro" between the two compartments. It is evident that the distribution and redistribution of etomidate between the vascular compartment and highly vascular tissues were slower in the pretreated group than in the control group.

Discussion

The major metabolic pathway of etomidate, (R)-(+)-ethyl-1(1-phenylethyl)-1H-imidazole-5-carboxylate, is hydrolysis of the ester to produce primarily (R)-(+)-1-(phenylethyl)-1H-imidazole-5-carboxylic acid. Oxidative N-dealkylation occurs to a slight extent, producing mandelic and benzoic acids, which are excreted in the urine. All the metabolic products are pharmacologically inert.[8]

It has been believed that etomidate is hydrolyzed at least in part in plasma. The earliest work on the assay of the drug** advocated the addition of fluoride ion to plasma samples containing etomidate to inhibit the plasma esterases and thus, hydrolysis of the drug. Quick recovery with absence of hang-over effects and the absence of cumulation with repeated doses of etomidate have also been explained by rapid hydrolysis in the serum and liver.[9] The question about the site of hydrolysis is also important, because if the drug were hydrolyzed in plasma, its duration of action would depend on pseudo-cholinesterase levels, and interactions with anti-cholinesterases and other drugs hydrolyzed in plasma would be possible. The present study demonstrates that etomidate is not significantly hydrolyzed in human or canine plasma.

The liver is the most probable site for biotransformation of the drug. BNPP, a specific inhibitor of the enzyme carboxylic acid hydrolase, which is found primarily in the liver, prolonged the duration of action of etomidate without affecting significantly its clearance from the plasma. At low hepatic extraction ratios, a metabolic inducer or inhibitor may profoundly affect the metabolic clearance of a drug, whereas at high extraction ratios, they may have only negligible effects.[10] Recovery from the hypnotic effect of etomidate occurs in the early distribution phase of the drug. The plasma level rapidly decreases, following injection of etomidate, as a result of its distribution into the tissues, and the drug is transferred rapidly out of the brain to maintain equilibrium.[3] BNPP pretreatment prolonged both the sleep time with etomidate and its distribution to and from the highly perfused organs. A drug that depresses the cardiovascular system can produce these effects. Further studies are needed to examine the effects of BNPP on the cardiovascular system.

** Janssen Pharmaceutica Research Products Information Service, Beerse, Belgium.

See NAPS Document No. 03362 for 2 pages of complete numerical data. Order from ASIS/NAPS, Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, New York 10017. Remit in advance for each NAPS accession number. Institutions and organizations may use purchase orders when ordering, however, there is a billing charge for this service. Make checks payable to Microfiche Publications. Photocopies are $5.00. Microfiche are $3.00 each. Outside the United States and Canada, postage is $3.00 for photocopy or $1.00 for fiche.

References

1. Morgan M, Lumley J, Whitwam JG: Etomidate, a new water-soluble non-barbiturate intravenous induction agent. Lancet 1:955–956, 1975
2. Ghoneim MM, Yamada T: Etomidate: A clinical and electroencephalographic comparison with thiopental. Anesth Analg (Cleve) 56:479–485, 1977
3. Van Hamme MJ, Ghoneim MM, Ambre JJ: Pharmacokinetics of etomidate, a new intravenous anesthetic. ANESTHESIOLOGY 49:274–277, 1978
4. Boyce JR, Wright FJ, Cervenko FW, et al: Prolongation of anesthetic action by BNPP (bis-[p-nitrophenyl]phosphate). ANESTHESIOLOGY 45:629–634, 1976
5. Garry PJ: Serum cholinesterase variants: Examination of several differential inhibitors, salts, and buffers used to measure enzyme activity. Clin Chem 17:183–191, 1971
6. Van Hamme MJ, Ambre JJ, Ghoneim MM: Mass fragmentographic determination of plasma etomidate concentrations. J Pharm Sci 66:1344–1346, 1977
7. Metzler CM: A User's Manual for NONLIN. Technical Report 7292/69/7292/005, The Upjohn Company, Kalamazoo, Michigan, 1969
8. Heykants JJP, Meuldermans WEG, Michiels LJM, et al: Distribution, metabolism, and excretion of etomidate, a short-acting hypnotic drug in the rat: Comparative study of (R)-(+) and (S)-(−) etomidate. Arch Int Pharmacodyn Ther 216:113–129, 1975
9. Kay B: A dose-response relationship for etomidate with some observation on cumulation. Br J Anaesth 48: 213–216, 1976
10. Gillette JR: Overview of factors affecting drug interactions. Ann NY Acad Sci 281:136–150, 1976

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/50/3/227/300331/0000542-197903000-00012.pdf by guest on 04 June 2023