Bioavailability of Sublingual Buprenorphine

John Mendelson, MD, Robert A. Upton, PhD, E. Thomas Everhart, PhD, Peyton Jacob III, PhD, and Reese T. Jones, MD

Buprenorphine administered sublingually is a promising treatment for opiate dependence. Utilizing a new, sensitive, and specific gas chromatographic electron-capture detector assay, the absolute bioavailability of sublingual buprenorphine was determined in six healthy volunteers by comparing plasma concentrations after 3- and 5-minute exposures to 2 mg sublingual and 1 mg intravenous buprenorphine. The amount of unabsorbed buprenorphine in saliva was measured after 2-, 4-, and 10-minute exposures to 2 mg sublingual buprenorphine in 12 participants. Pharmacokinetic parameters were analyzed by analysis of variance; bioequivalence was evaluated by the Schuirmann two-sided test. The 3- and 5- minute sublingual exposures each allowed 29 ± 10% bioavailability (area under the plasma concentration–time curve unextrapolated) and were bioequivalent. Buprenorphine recovered from saliva after 2-, 4-, and 10-minute exposures was, on average, 52% to 55% of dose. Increased saliva pH was correlated with decreased recovery from saliva. Study results indicate that bioavailability of sublingual buprenorphine is approximately 30%. Sublingual exposure times between 3 and 5 minutes produce equivalent results. Buprenorphine remaining in saliva causes an almost twofold overestimation of bioavailability.

Buprenorphine is a synthetic, lipophilic, potent (20–40 times greater analgesic potency than morphine) oripavine opiate analgesic effective in the treatment of opiate dependence.[1–3] Low oral bioavailability (approximately 14%),[4] caused largely by hepatic first-pass metabolism, makes sublingual administration an attractive alternative for treatment. In clinical trials, buprenorphine was administered as a 30% ethanol solution with participants retaining the dose sublingually for up to 10 minutes. Assessment of the pharmacokinetics of buprenorphine has been hampered by the difficulty in quantifying low plasma levels.[5] By measuring the amount of buprenorphine remaining in saliva after 2.5 or 10 minutes of sublingual exposure, a prior study inferred a sublingual bioavailability of 25% to 50%.[6] Differences in the amount of buprenorphine recovered from saliva with increased exposure were not evident.

Using a recently developed, sensitive, and specific gas chromatographic electron-capture detector (GC-ECD) assay for buprenorphine in plasma, absolute bioavailability of sublingual buprenorphine was estimated by comparing the plasma concentrations achieved with those from an intravenous dose. Sublingual exposure times of 3 and 5 minutes for bioavailability of buprenorphine were compared using the plasma-based method. In a separate study, those bioavailabilities were compared with estimates from a less direct method based upon the amount of buprenorphine recovered in saliva after exposures of 2, 4, and 10 minutes.

MATERIALS AND METHODS

Subjects

Six healthy volunteers (five men, one woman), 21 to 38 years of age (mean ± SD = 29 ± 6 years), partici-

From the Drug Dependence Research Center, Langley Porter Psychiatric Institute (Drs. Mendelson, Upton, Everhart, Jacobs, and Jones), the Department of Biopharmaceutical Sciences (Dr. Upton), and the Division of Clinical Pharmacology and Experimental Therapeutics (Dr. Jacob), University of California, San Francisco, San Francisco, California. Supported in part by United States Public Health Service grants DA01696 and DA00053 and contract No. 271-90-7307 from the National Institute on Drug Abuse, National Institutes of Health. Submitted for publication July 20, 1996; accepted in revised form October 16, 1996. Address for reprints: John Mendelson, MD, Drug Dependence Research Center, Langley Porter Psychiatric Institute, University of California, San Francisco, 401 Parnassus Avenue, San Francisco, CA 94143-0984.

TABLE I

Inclusion and Exclusion Criteria for Bioavailability and Saliva Recovery Studies

Inclusion Criteria
Male or female and between 21 and 40 years of age
Experienced in the use of illicit opiates
In good health with normal physical examination and laboratory screening test results
Within ±15% of ideal body weight
Without oral cavity pathologic conditions
Women must have a negative urine pregnancy test result before each experimental session and must use barrier birth control methods until completion of the study
Exclusion Criteria
A history of clinically significant medical or psychiatric disorders
Opiate-dependence, as defined by the DSMIII-R criteria, or dependence on other psychoactive drugs other than nicotine or caffeine
Known hypersensitivity to buprenorphine or other opiate-like analgesic agents
Current treatment with any prescription medication

DMSIII-R, Diagnostic and Statistical Manual, Third Edition (Revised).

pated in the bioavailability study. Twelve similar volunteers (nine men, three women), 21 to 40 years of age (mean ± SD = 32 ± 6 years), participated in the saliva recovery study. Inclusion and exclusion criteria for both studies are in Table I. Written, informed consent was obtained. The protocols were approved by the Committee on Human Research, University of California, San Francisco.

All participants were studied as outpatients. Each dose was administered after an overnight fast and a 12-hour requested abstinence from psychoactive drugs (including nicotine and caffeine). The participants were not allowed to drink fluids or smoke cigarettes for 1 hour after drug administration. A low-fat lunch was provided 4 hours after administration.

Study Design

The data presented come from two separate studies. For the plasma-based bioavailability study, six participants were administered buprenorphine at approximate weekly intervals under the following experimental conditions: 2 mg buprenorphine in 30% ethanol solution held sublingually for 3 minutes, 2 mg buprenorphine in 30% ethanol solution held sublingually for 5 minutes, and 1 mg buprenorphine by intravenous infusion. The sublingual conditions were randomized in sequence. The intravenous infusion was always administered between the sublingual treatments.

For the saliva recovery study, 12 participants were studied in a 3 × 3 balanced Latin Square design, with at least 3 days between sessions, under the following conditions: 2 mg buprenorphine in 30% ethanol solution held sublingually for 2 minutes, 2 mg buprenorphine in 30% ethanol solution held sublingually for 4 minutes, and 2 mg buprenorphine in 30% ethanol solution held sublingually for 10 minutes.

Medications

Buprenorphine hydrochloride was supplied by the National Institute on Drug Abuse, prepared as 2 mg/mL in 30% ethanol solution. A 3.3-mL aliquot of commercially available buprenorphine injection solution (Buprenex in 0.3 mg/mL ampules, Reckitt and Colman Products, Ltd.; United Kingdom) was diluted to 30 mL with 0.9% NaCl, and used for the intravenous dose.

Dose Administration and Sample Collection

The intravenous dose of buprenorphine was infused into a forearm vein at a rate of 1 mL/min under syringe pump control over a 30-minute period. Sublingual doses were administered with a 1-mL tuberculin syringe. The buprenorphine solution was placed in the right posterior sublingual area at the base of the tongue. Participants did not swallow after administration until instructed to do so by an observer. In the bioavailability study, when instructed, participants swallowed once to terminate exposure and thereafter swallowed *ad libitum*. In the saliva recovery study, sublingual exposure was terminated by spitting the remaining solution and accumulated saliva into a preweighed 30-mL centrifuge tube. Two separate rinses of 35 mL of distilled water were then swirled around the oral cavity for 30 seconds each and collected in a preweighed jar. Saliva pH was measured before and after administration with a microprobe electronic pH meter (Lazar Research Lab; Los Angeles, CA). Samples were frozen at −20°C until analyzed.

Plasma samples (10 mL) were obtained through an intravenous catheter in the forearm of the nondominant hand. Samples were collected before buprenorphine and at 5, 20, 30, and 40 minutes, and 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours after administration.

Determination of Buprenorphine Concentration in Plasma

The assay involved a three-step extraction of analyte from 1.0 mL of plasma sample spiked with 50 ng

of internal standard (N-n-propylnorbuprenorphine). Buprenorphine and internal standard in the extract were converted to the heptafluorobutyryl derivatives using heptafluorobutyric anhydride, excess reagent was removed under vacuum, and the residues were reconstituted in 20 μL of n-butyl acetate. Samples (3 μL) were analyzed by gas chromatograph using a 25 m × 0.2 mm (internal diameter) fused silica capillary column, splitless injection, and electron-capture detection. Results were quantitated by measuring chromatographic responses of a series of calibration standard samples prepared with each run.

Calibration curves were linear from 0.1 ng/mL to 20 ng/mL. The limit of quantitation was 0.1 ng/mL for all except two of the runs (which were 0.3 ng/mL and 0.2 ng/mL). Accuracy and precision of the method were such that replicate assays of spiked control samples at 0.1 ng/mL and 0.2 ng/mL, or 0.15, 0.5, 2.0, 5.0, 10, or 15 ng/mL, assayed concurrently with study samples, had coefficients of variation ranging from 3.97% to 18.47%, and a bias of only −8.6% to +9.4% (for each control, n = 1−3 per run).

Determination of Buprenorphine Concentration in Saliva

Saliva samples were diluted one hundredfold and mouth-rinse samples tenfold with 0.01 mol/L sulfuric acid. Aqueous standards were used neat. One milliliter of sample, 100 μL of 30 μg/mL N-n-pentylnorbuprenorphine, 0.5 mL of 1 mol/L NaOH, and 2 mL of ethyl acetate/heptane (4:1 vol/vol) were combined, vortexed 5 minutes, centrifuged at 5,000 *g* for 10 minutes, and the aqueous phase frozen by immersion in a dry ice and acetone bath. The organic phase was then decanted, the aqueous phase reextracted with another 2-mL aliquot of ethyl acetate/heptane (4:1 vol/vol) as described above, and the second organic extract added to the first. The combined organic extracts were evaporated to dryness, reconstituted in 0.5 mL of high-performance liquid chromatography mobile phase, and 25-μL aliquots were injected via autosampler into the high-performance liquid chromatograph.

The high-performance liquid chromatography system consisted of an autosampler (Model WISP 7100, Waters Associates; Milford, MA), Shimadzu pump (Model LC-6A, Shimadzu Corp.; Kyoto, Japan), ultrasphere ODS column (C_{18}, average particle diameter 5 μ, 4.6 × 25 cm) (Model 235329, Beckman Instruments; Fullerton, CA), fluorescence detector (Shimadzu model RF-535), and Hewlett-Packard integrator (Model 3397A, Hewlett-Packard; Wilmington, DE). The mobile phase was acetonitrile/0.1% phosphoric acid in a 40:60 ratio, with pH adjusted to 3.0 with aqueous NaOH. The flow rate was 1 mL/min. The detector excitation and emission wavelengths were 285 nm and 355 nm, respectively. Retention times for buprenorphine and N-n-pentylnorbuprenorphine were ~4.8 and ~9.8 minutes, respectively. Norbuprenorphine, buprenorphine's major metabolite, had a retention time of ~3.1 minutes and was not detected in saliva or mouth-rinse samples.

Buprenorphine standard samples were prepared by diluting buprenorphine HCl in 0.01 mol/L of sulfuric acid. Ten or more standards, spanning the range from 0 μg/mL to 10 μg/mL, were included with each batch of samples and used to construct a standard curve based on peak area ratios of buprenorphine and N-n-pentylnorbuprenorphine. Standard curves were linear in the range of 0 μg/mL to 10 μg/mL. Control samples, prepared by spiking blank saliva and mouth rinse, at concentrations spanning the expected concentration ranges, were included in each batch of samples.

N-n-propylnorbuprenorphine was synthesized in the laboratory by reductive alkylation of norbuprenorphine with propionaldehyde and sodium borohydride and was converted to hydrochloride salt and crystallized from ethanol solution by addition of ether. Thin layer chromatography (TLC) revealed complete conversion of norbuprenorphine to the propyl derivative. N-n-pentylnorbuprenorphine was prepared in an analogous manner from norbuprenorphine and n-pentanal, and was used as the free base.

Calibration curves were linear from 0.2 μg/mL to 30 μg/mL. The limit of quantitation was 0.2 μg/mL. Accuracy and precision of the method were such that replicate assays of spiked control samples at 0.2, 2, 5, and 30 μg/mL had coefficients of variation ranging from 2.63% to 6.92% and a bias of 2% to 4% (for each control, n = 5 per run).

Pharmacokinetic Analysis

The area under the plasma concentration−time curve (AUC) was estimated from the time of administration ($t = \varnothing$) to the time of the last assayed sample ($t = t_z$), using the trapezoidal equation for periods of increasing or stationary concentration, and the logarithmic-trapezoidal equation for periods of decreasing concentration.[7] This area was extrapolated from t_z to infinity ($AUC_{0-\infty}$) by dividing the last concentration measured by an estimate of the terminal log-linear slope. The terminal slope was estimated, for purposes of extrapolating AUC, from a least-squares linear fit (unweighted) to the last three time points of the plasma concentration−time curve (semilogarithmic). The peak plasma concentration (C_{max}) was taken as the concentration in the plasma sample hav-

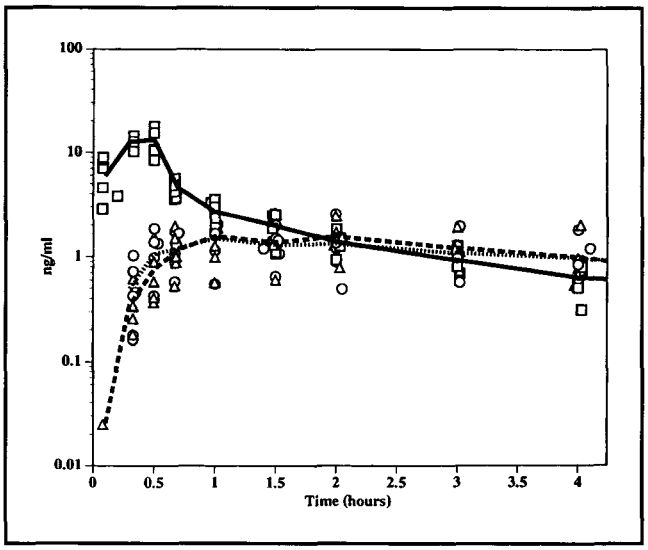

Figure 1. Buprenorphine in plasma after 1-mg intravenous dose (solid line, mean; □, individual values), and 2-mg sublingual doses: 3-minute exposure (dotted line, mean; ○, individual values); 5-minute exposure (dashed line, mean; △, individual values); n = 6 for all doses.

ing the highest concentration. No attempt was made to interpolate concentrations between sampling times. The total amount of buprenorphine base remaining in the saliva and in each of the two rinses was determined. The values for saliva plus first and second rinses were summed.

Drug Effect Measures

Heart rate and systolic and diastolic blood pressure were measured with a cardiovascular monitor (Model VSM-2, Physio-Control Corp.; Redmond, WA). Respiratory rate was measured by counting the number of inhalations per minute. Verbal ratings of global intoxication on a 0 to 100 scale, with 0 representing no effect and 100 the maximum effect experienced after opiate drugs were obtained in both studies. Additional measures of subjective drug effects were obtained in the bioavailability study using self-ratings of symptom intensity on three subscales measuring euphoria, sedation, and dysphoria (MBG, PCAG, LSD) from the Addiction Research Center Inventory,[8] a 31-item, adjective-rating checklist consisting of opiate agonist and antagonist symptoms, visual analog scales (range, 0–100) measuring "good" drug effect, "bad" drug effect, "high," drunkenness, sickness, and hangover, and the Profile of Mood Scale.[9] The self-ratings were obtained before drug administration and at 1, 2, 4, 6, 8, 10, and 12 hours after administration in the bioavailability study and at 1, 2, 3, 4, 5, 6, and 7 hours in the saliva recovery study.

STATISTICAL ANALYSIS

Statistical analyses were performed on AUCs, C_{max}, and peak time (t_{max}) using the Statistical Analysis System (SAS; version 6.10) program (SAS Institute, Cary, NC). The t_{max} was analyzed untransformed, and AUC and C_{max} as their logarithmic (natural) transforms, divided by the varying dose sizes. For AUC from all three doses, dose was analyzed as an intersubject effect and sequence as an intrasubject effect, with the dose-by-sequence interaction being evaluated as a surrogate for period effect (because of the limited number of degrees of freedom). Peak concentrations and t_{max}s were compared by analysis of variance (ANOVA) between the two sublingual treatments only, allowing dose and period each to be analyzed as intersubject effects and sequence as an intrasubject effect.

Bioequivalence between the two sublingual treatments, measured by AUC, C_{max}, and t_{max}, was evalu-

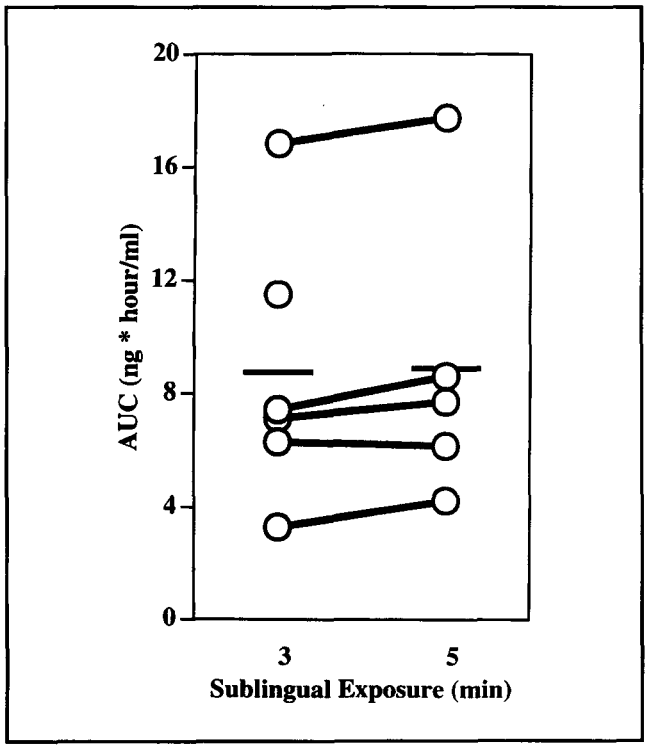

Figure 2. Individual areas under the concentration–time curve (AUC) for 3- and 5-minute sublingual exposures (○—○, one individual).

TABLE II

Pharmacokinetics of Buprenorphine Measured in Plasma

Pharmacokinetic Parameters	3-minute SL Dose A, n = 6 (2 mg)	5-minute SL Dose B, n = 5 (2 mg)	IV Infusion Dose C, n = 6 (1 mg)	Statistical *P* Value for Treatment Effect (Contrasts)	Ratio 3-minute SL:IV	Ratio 5-minute SL:IV	Ratio 5-minute SL: 3-minute SL
AUC unextrapolated (hr · ng/mL)	8.75 ± 4.75	8.89 ± 5.22	14.7 ± 3.5	0.0001 (A = B, A ≠ C, B ≠ C)	0.28 ± 0.10	0.29 ± 0.10	1.11 ± 0.12
AUC extrapolated (hr · ng/mL)	14.3 ± 8.7	13.2 ± 8.8	18.4 ± 6.5	0.0002 (A = B, A ≠ C, B ≠ C)	0.36 ± 0.13	0.33 ± 0.13	0.95 ± 0.18
C_{max} (ng/mL)	1.60 ± 0.66	1.72 ± 0.87	14.3 ± 3.0	0.114*			1.13 ± 0.12
t_{max} (hr)	1.25 ± 0.42	1.62 ± 0.55	0.44 ± 0.09	0.474*			0.32 ± 0.84†
Cl (L/hr)			62.5 ± 21.8				
$t_{1/2}$ (hr)‡			16.2 ± 20.1				

* *Analysis of variance for peak concentration and peak time, performed on the data from the sublingual treatments only.*
† *Difference, 5-minute sublingual minus 3-minute sublingual.*
‡ *Approximate estimate limited by assay sensitivity considerations.*
Values are presented as the mean ± standard deviation. SL, sublingual; IV, intravenous; AUC, area under the concentration–time curve; C_{max}, peak concentration; t_{max}, time to C_{max}; Cl, clearance; $t_{1/2}$, elimination half-life.

ated using the two one-sided (a = 0.05) confidence intervals (Schuirmann) tests. The mean square error term for all three variables in this analysis derived from a two-sample ANOVA that compared the sublingual treatments only and included a period effect. Subjective drug effects were analyzed by ANOVA.

Saliva concentration data were also analyzed by ANOVA, with sublingual exposure time as the intersubject factor and exposure sequence as the intrasubject factor. The relationship between saliva pH and recovery of buprenorphine in saliva was investigated by linear correlation analysis of data pooled across all three exposure times.

RESULTS

Pharmacokinetics in Plasma

Mean plasma concentrations of buprenorphine are shown in Figure 1; unextrapolated AUCs for each participant are shown in Figure 2; pharmacokinetic parameter estimates are shown in Table II.

The two sublingual doses each had smaller AUCs (after adjustment of dose) than did the intravenous dose ($P < 0.0002$), whether AUC was estimated unextrapolated or extrapolated. No AUC, C_{max}, or t_{max} differences were evident between the two sublingual exposure times. With each AUC and peak concentration, bioequivalence between the two sublingual treatments was confirmed by two one-sided confidence intervals (Schuirmann) tests. Bioequivalence for the 3-minute treatment was within 80% to 125% of that for the 5-minute treatment ($P \leq 0.05$). No significant sequence or period effect (dose-by-sequence interaction) was evident.

Pharmacokinetics in Saliva

The amount of buprenorphine remaining in saliva and mouth rinses was not significantly different after the 2-, 4-, and 10-minute exposures (1.01 ± 0.5 mg, 0.97 ± 0.5 mg, and 0.98 ± 0.3 mg, respectively, corresponding to 55 ± 26%, 52 ± 25%, and 53 ± 15% of the dose; Table III). No significant sequence effect or period effect (dose-by-sequence interaction) was seen. Saliva pH was significantly but not closely correlated with recovery of buprenorphine (Figure 3). With increasing saliva pH, less buprenorphine was recovered ($r = -0.33$, $P = 0.05$ saliva alone; $r = -0.40$, $P = 0.02$ saliva plus mouth rinses).

Drug Effect Measures

No significant differences were evident in any drug effect measures in either experiment.

DISCUSSION

The absolute bioavailability (Cl/F) of buprenorphine from a sublingual solution dose in ethanol was 28% to 36%. Sublingual holding times between 3 and 5 minutes were bioequivalent in the extent of bioavailability that resulted. Although differences in t_{max} could not be established statistically between 3- and 5-minute treatments, bioequivalence between the two treatments could not be established either. How-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.