Pilocarpine Disposition and Salivary Flow Responses Following Intravenous Administration to Dogs

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Oral doses of pilocarpine increase salivary flow rates in patients afflicted with xerostomia (dry mouth). This study examined the pharmacokinetics of and a pharmacodynamic response (salivation) to intravenous pilocarpine nitrate administration in dogs. Disposition was linear over a dose range of 225–600 μg/kg; plasma concentrations were 10–120 μg/L. Elimination was rapid and generally biphasic, with a terminal elimination half-life of approximately 1.3 hr. The systemic clearance of pilocarpine was high (2.22 ± 0.49 L/kg/hr) and its steady-state volume of distribution (2.30 ± 0.64 L/kg) was comparable to that of many other basic drugs. All doses of pilocarpine induced measurable submaxillary and parotid salivary flow rates which could be maintained constant over time. Cumulative submaxillary saliva flow was linearly related to total pilocarpine dose. Plasma pilocarpine concentration was linearly related to both steady-state and postinfusion submaxillary salivary flow rates.

KEY WORDS: xerostomia; pilocarpine; pharmacokinetics; pharmacodynamics.

INTRODUCTION

Dryness of the mouth resulting from diminished production of saliva (xerostomia) is a clinically important symptom experienced by millions of patients as a side effect of drug therapy or head/neck irradiation or in association with Sjogren's syndrome, a complex of symptoms experienced by a significant percentage of patients with autoimmune diseases. Patients experience difficulties eating, speaking, swallowing, and tolerating dentures (1–5). They frequently develop oral and pharyngeal candidiasis and severe tooth decay (5). These often lead to further debility, risk of serious head and neck infections, and deterioration in the quality of life.

Pilocarpine is a cholinomimetic agent available only as ophthalmic dosage forms for glaucoma treatment. Several investigations have shown that pilocarpine has promise as a salivary stimulant when orally administered to normal volunteers (6,7), patients with xerostomia secondary to radiation therapy (7–9), and those with salivary gland dysfunction and/or Sjogren's syndrome (10). Oral pilocarpine has a short duration of action and can produce numerous unpleasant systemic side effects, including excessive sweating, nausea, vomiting, and diarrhea. If it is to be used effectively in the treatment of xerostomia, it should be administered by a route and at a controlled rate to optimize the salivary response and minimize untoward effects. Pharmacokinetic/pharmacodynamic information is necessary to design such inputs. To date there are no published assays quantifying pilocarpine in biological fluids other than aqueous humor (11), and the pharmacokinetic behavior of pilocarpine following any route of administration other than intraocular has not been elucidated in any species.

MATERIALS AND METHODS

Animals

Six female Beagle dogs (age, 0.75–4.0 years; weight, 8.4–10.9 kg; Marshall Farms, North Rose, NY) were housed in individual cages, fed a standard diet (Prolab 200 Canine Diet, Agway, Waverly, NY), and allowed water ad libitum.

Drugs and Reagents

Pilocarpine nitrate, clonidine hydrochloride, sodium fluoride (Sigma Chemical, St. Louis, MO), and isopilocarpine nitrate (Aldrich Chemical) were all used as received. Pilocarpine nitrate was assayed (12) for isopilocarpine contamination and determined to be >99% pure. Sodium pentobarbital (64 mg/ml; Fort Dodge, Fort Dodge, IA) and atropine (1 mg/ml, Elkin-Sinn, Cherry Hill, NJ) were sterile solutions. Acetonitrile, methanol, methylene chloride, and diethyl ether (J. T. Baker, Phillipsburg, NJ), as well as mono- and dibasic potassium phosphate (Fisher Scientific, Springfield, NJ), were HPLC-grade reagents and used without further purification. Water was distilled and deionized (Milli-Q purification system, Millipore Corp., Bedford, MA). All glassware was borosilicate and silanized with a 1:100 solution of Prosil-28 (PCR, Gainesville, FL) to minimize adsorptive loss of drug.

Intravenous pilocarpine nitrate infusions were prepared in normal saline from aqueous stocks made within 2 hr of use. They were individualized to each dog's body weight and concentration-adjusted (0.43–1.48 mg/ml) so that each delivery rate was achieved using a 1 ml/min infusion rate.

Protocol, Drug Administration, and Sample Collection

Dogs were anesthetized with an intravenous bolus dose of sodium pentobarbital (30–50 mg/kg, administered slowly, to effect) followed by infusion at a rate just sufficient to prevent skeletal muscle movements other than those associated with ventilation (approximately 30 mg/hr). The airway was maintained by a 7-mm cuffed endotracheal tube.

Heparinized catheters were inserted into both cephalic and the right saphenous veins. Pilocarpine and pentobarbital were delivered via the right cephalic vein. Blood was sampled from the left cephalic vein. Normal saline was infused via the saphenous vein to maintain baseline hematocrit.

Left and right Wharton's (submaxillary) and Stenson's (parotid) ducts were cannulated with Teflon catheters (22 G,

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1 in.), connected to polyethylene tubing (12 in., 0.86-mm i.d.) draining into saliva collection tubes.

Pilocarpine was administered at 1.0 ± 0.05 ml/min (IMED Infusion Pump, IMED Corp., San Diego, CA) at doses of 90, 162, and 240 μg/kg/hr for 2.5 hr. A randomized, crossover design with at least 1 week between treatments was used for each dog. In addition, an intermediate infusion rate (195 μg/kg/hr) was administered to two animals to provide more data on pharmacokinetic linearity.

For the first six experiments, 2-ml blood samples were drawn into heparinized tubes, and hematocrits measured at 0, 15, 45, 75, 105, 125, 135, 145, 151, 158, 165, 175, 195, and 235 min after the start of each infusion. Subsequently samples were drawn at 0, 8, 15, 35, 55, 75, 95, 115, 135, 145, 155, 165, 175, 185, 205, 225, and 245 min to provide more data during the distributive phase. Samples were immediately placed in silanized glass tubes (13 × 100 mm) containing 250 mg of solid NaF (to retard esterase-mediated degradation of pilocarpine), agitated, centrifuged (3 min, 13,000 *g*), and plasma transferred to polyethylene tubes containing additional NaF (200 mg). Tubes were sealed, maintained at 0°C during the experiment, then stored at −20°C until assay.

Submaxillary and parotid saliva samples were collected over 10-min periods throughout the experiment until basal salivary flow rates were reestablished. Saliva was stored at room temperature in sealed tubes until quantitated with calibrated pipettes (samples >1 ml) or Hamilton syringes (samples <1 ml).

At the conclusion of each experiment atropine (0.5 mg) was administered subcutaneously and animals were monitored until the swallow reflex returned. All procedures were approved by the University of Connecticut Health Center Animal Care Committee.

Pilocarpine Assay

Sample Preparation. Aqueous stock solutions of pilocarpine nitrate and clonidine hydrochloride (internal standard) were stored in the dark at 4°C. They were stable for at least 1 month. Working pilocarpine solutions (1–10 mg/L) were prepared daily.

One-half milliliter of plasma, 0.5 ml of clonidine solution (30 μg/L in 50 m*M* potassium phosphate buffer, pH 9.0), and 3.0 ml of methylene chloride were placed in 13 × 100-mm glass tubes, capped, shaken (200 osc/min, 10 min), and centrifuged (2100 *g*, 10 min). The organic phase was transferred to a 15-ml glass centrifuge tube and evaporated at 40°C under nitrogen. The residue was reconstituted in 0.15 ml of 0.001 *N* HCl and vortexed with diethyl ether (2.0 ml) for 2 min. The phases were separated by centrifugation and the ether phase discarded. Most residual ether was removed by applying vacuum for 10 sec. Samples were frozen (−20°C) overnight. Upon thawing, vacuum was reapplied to remove traces of ether. The final volume was approximately 110 μl, of which 35–100 μl was injected onto the analytical column.

Chromatography. Samples were analyzed using a Varian Model 5020 high-pressure liquid chromatograph (Varian Associates, Palo Alto, CA) with a manual injector, 214-nm fixed-wavelength UV detector (Models U-6K and 441, respectively, Waters Associates, Milford, MA), and recorder (Varian Model 9176, 5-mV full-scale). Separations were achieved at room temperature with a 150 mm × 4.6-mm Spherisorb ODS-1 column (5 μm; Alltech, Deerfield, IL) protected by a 1.5-cm guard column filled with 37 μm C-18 Corasil (Waters Associates). The mobile phase was potassium phosphate (7 m*M*, pH 4.0), acetonitrile, and methanol (55:30:15) flowing at 1.1 ml/min.

Calibration and Reproducibility. Pilocarpine and clonidine had retention times of 7.2 and 12 min, respectively, and were resolved from pentobarbital. Pilocarpine and its epimer, isopilocarpine, coeluted. Standard curves of peak height ratio versus concentration were linear over the concentration range of 10 to 300 μg/L ($r > 0.99$; absolute pilocarpine recovery, >90%). The lower quantifiable limit for pilocarpine (S/N > 4) was 10 μg/L. Coefficients of variation were 4.3 and 1.7% ($n = 5$) at 30 and 300 μg/L and 4.9 and 2.7% ($n = 5$) at the same concentrations for intra- and interday determinations, respectively. The long-term stability of plasma pilocarpine mixed with NaF stored at −20°C was examined by periodically reassaying plasma samples from experiments. Such samples reassayed to within 10% of their original concentration when stored for up to 3 months.

Data Analysis

Pharmacokinetics. Plasma concentration–time data were described by one- and two-compartment linear models with zero-order input into, and first-order elimination from, the central compartment. An equation and initial parameter estimates were obtained from graphical inspection of the data and refined using the iterative least-squares regression program PCNONLIN (13). Data were weighted equally or as the reciprocal of their value. Fits were evaluated by randomness of scatter, weighted residual sum of squares, and visual inspection of plots of weighted residuals vs time. An *F* test was used to evaluate significant improvements in fit of data which were well fitted by both a one- and a two-compartment model (14).

The equation of a one-compartment model best fit the pilocarpine pharmacokinetics for the 405 μg/kg dose to dog 3. All other data were best fit by two-compartment equations. Central compartment, steady-state, and β-elimination phase volumes of distribution (V_c, V_{ss}, and V_β, respectively), systemic clearance (Cl), and elimination half-life ($t_{1/2}$-β), were calculated from the coefficients and exponents of the fitted equations according to Gibaldi and Perrier (15). In addition, C_p^{ss} values were calculated as the ratio of intravenous infusion rate to clearance. When plasma concentrations appeared to reach steady state within the infusion period, the empirical and calculated values of C_p^{ss} were compared and, in all cases, agreed within ±5%.

Pharmacodynamics. To model steady-state and postinfusion salivary flow rate data as a function of time and pilocarpine concentration, the following relationship was used:

$$E = (a)\,(C_p) \qquad (1)$$

where

E = submaxillary salivary flow rate
C_p = plasma pilocarpine concentration
a = slope

To analyze the steady-state data, Eq. (1) was fitted to the combined data from all doses and dogs. Postinfusion data were analyzed by preparing composite plots of E as a function of C_p for all doses in each dog and fitting Eq. (1) to each set of data. While salivary flow in the absence of pilocarpine was not detected in any experiment, a line fitted to Eq. (1) may have a nonzero y intercept. This would be expected if the data were from a linear region of a curvilinear relationship where the curvature occurring at low drug concentrations was not observed. Fits were, therefore, not forced through zero; intercepts were statistically compared with zero.

Statistical Analysis. A three-way analysis of variance (ANOVA) was used to test for treatment, period, and subject differences among means. Tukey's standardized range test was used to delineate those differences. The statistical package SAS was used (16).

RESULTS

Throughout each experiment cardiac and respiratory rates of the animals remained stable at approximately 130 beats and 30 breaths/min, respectively. Responses to pilocarpine appeared to be dose dependent and included salivation, bronchial and intestinal secretion, and, occasionally, urination and defecation (never diarrhea). Fluid losses, as judged by changing hematocrit, varied with dose, necessitating the administration of 0.2–1.25 L of normal saline during the experiments to maintain a stable hematocrit. In the extreme, replacement volumes approached four times the total volume of saliva secreted.

Figure 1 shows plasma pilocarpine concentration versus time and associated submaxillary salivary flow rate versus time profiles for three infusion rates in a representative dog. Rapid initial increases in plasma concentrations, followed by more moderate increases as steady state was approached, were observed. Submaxillary salivary flow profiles generally paralleled plasma concentration–time curves, although the pronounced biphasic decline in postinfusion plasma levels was not observed with the salivary flow rate data. Usually, steady-state flow rates were achieved. In such cases, flow was stable over time, with no indication of tachyphylaxis, even though flow rates as high as 1.30 ml/min were induced.

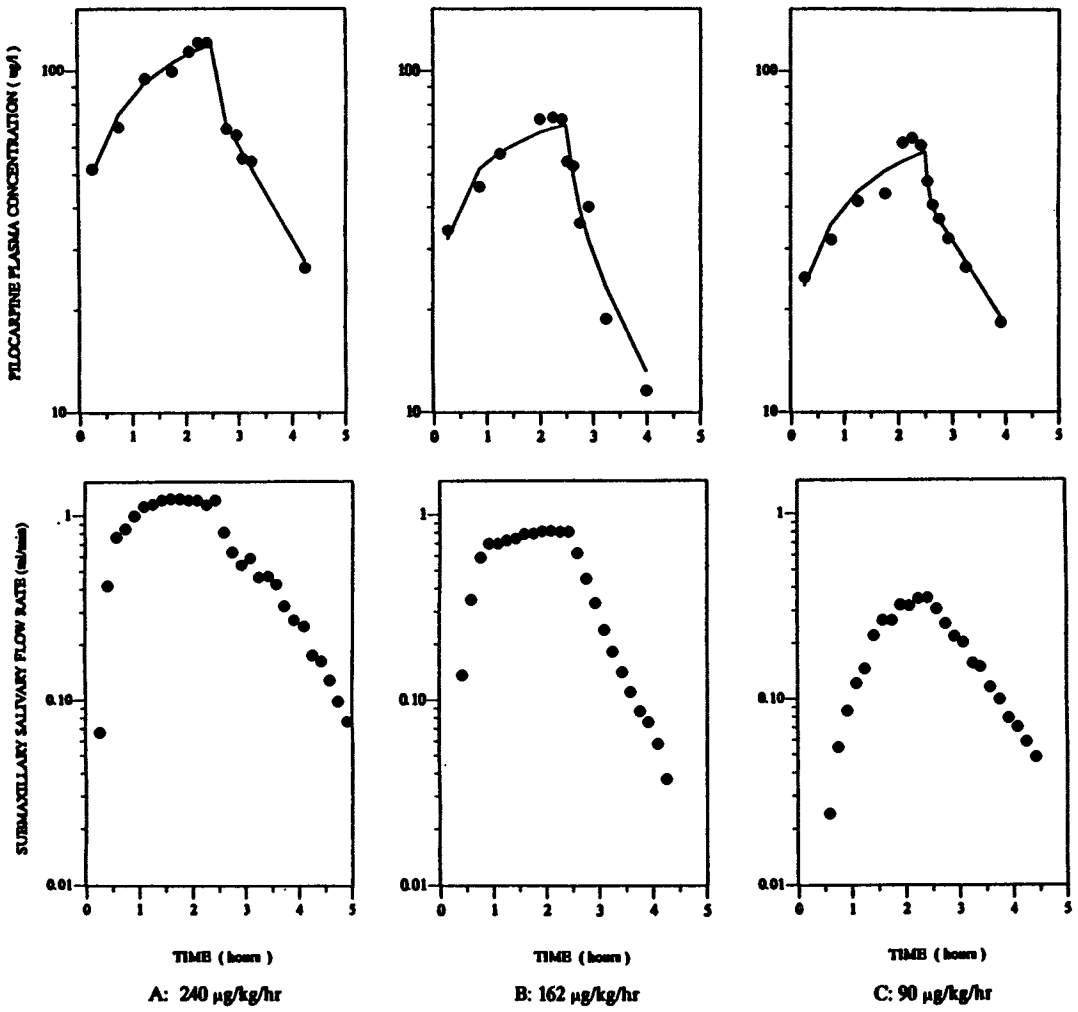

Fig. 1. Semilogarithmatic relationships between pilocarpine plasma concentrations (top) and submaxillary gland salivary flow rates expressed per gland pair (bottom) as functions of time for three intravenous infusion rates of 2.5-hr duration in dog 2.

Table I. Summary of Canine Pharmacokinetic Parameters Determined from Intravenous Pilocarpine Infusions

Dog	k_{10} (hr^{-1})	k_{12} (hr^{-1})	k_{21} (hr^{-1})	$t_{1/2}$-β (hr)	V_c (L/kg)	V_{RS} (L/kg)	V_β (L/kg)	Cl (L/kg/hr)
1	5.15	8.04	1.74	1.18	0.361	2.04	2.95	1.73
2	5.04	11.60	2.09	1.07	0.442	1.88	2.56	1.71
3	6.89	7.23	1.54	1.06	0.400	2.27	3.94	2.69
4	6.71	10.20	2.30	1.52	0.507	3.44	5.17	2.84
5	5.41	8.44	1.62	1.74	0.558	2.73	3.14	2.58
6	6.01	12.40	2.12	1.14	0.414	1.46	2.65	1.78
Mean	5.87	9.65	1.90	1.29	0.447	2.30	3.40	2.22
(SD)	(0.73)	(1.90)	(0.28)	(0.26)	(0.067)	(0.64)	(0.91)	(0.49)

Mean calculated steady-state plasma concentrations over all dogs were 48.4 ± 18.0, 73.6 ± 13.4, 76.6 ± 20.6, and 109.0 ± 20.8 μg/L for the 90, 162, 195, and 240 μg/kg/hr infusion rates, respectively. Post infusion declines were generally biexponential, with rapid distributive phases and a mean terminal half-life of 1.29 ± 0.26 hr.

Table I summarizes the pharmacokinetic parameters. The systemic clearance of pilocarpine (2.22 ± 0.49 L/kg/hr) exceeded published values of mean canine liver blood flow [1.38 L/kg/hr (17)], consistent with plasma- and/or extrahepatic tissue esterase-mediated hydrolysis of pilocarpine. The steady-state volume of distribution (2.30 ± 0.64 L/kg) is comparable to that of many other weak bases including clonidine, metoprolol, morphine, and procainamide (18). Statistical analysis showed systemic clearance to be independent of dose, dog, and period, consistent with linear pharmacokinetics.

Basal parotid and submaxillary salivary flow rates under anesthesia were undetectable in all dogs. Mean cumulative submaxillary saliva volumes ranged from 51.5 ± 23.7 ml at the lowest dose to 173 ± 28.0 ml at the highest (Table II). Corresponding parotid volumes were substantially lower (12.9 ± 6.1 and 65.2 ± 29.6 ml, respectively) and more variable. Both submaxillary and parotid saliva volumes were linear functions of dose, with regression coefficients of 0.902 and 0.695, respectively. Submaxillary flow rate (hereafter referred to as "salivary flow rate") was selected as the response variable for pharmacodynamic modeling.

Steady-state salivary flow rates increased linearly with plasma concentration ($r = 0.76$), with an intercept that was not statistically different from zero ($P = 0.27$) (Fig. 2). Normalizing the abscissa for each animal's baseline state of hydration did not reduce the variance.

Postinfusion salivary flow rates were linearly related to plasma concentrations in each dog (Fig. 3). The slopes obtained (Table III) suggested a similar degree of responsiveness to pilocarpine among dogs. One intercept (dog 4) statistically differed from zero. The twofold range in the slopes corresponded to the range of total saliva output by individual dogs.

A different function described the salivary flow rate versus pilocarpine plasma concentration relationship prior to achieving steady state. These plasma concentrations were not associated with the same values of salivary flow rate as identical postinfusion plasma concentrations.

DISCUSSION

The anesthetized dog was selected as the experimental model in this study. Its veins and salivary ducts are easily cannulated, enabling continuous saliva collection and intensive blood sampling. The present study was made possible by the development of an assay for pilocarpine in plasma using samples in which esterase activity was inhibited with sodium fluoride to permit storage for reasonable time periods prior to assay. Using this assay, we believe that several important observations have been made concerning the disposition of pilocarpine and its relationship to salivary flow induction in the dog.

Pilocarpine disposition in these dogs is linear over a

Table II. Total Submaxillary and Parotid Saliva Volumes Associated with Intravenous Infusions of Pilocarpine

	Total submaxillary and parotid saliva volume (ml) at							
	600 μg/kg		495 μg/kg		405 μg/kg		225 μg/kg	
Dog	Submaxillary	Parotid	Submaxillary	Parotid	Submaxillary	Parotid	Submaxillary	Parotid
1					128	320	75.8	22.7
2	189	112.0			110	7.2	42.4	15.5
3	159	37.9	164	20.3	124	12.3	34.5	5.1
4	218	88.0	142	46.1	139	46.0	49.5	15.1
5	165	40.3			122	27.5	17.4	5.5
6	135	49.7			81.4	32.9	86.7	26.1
Mean	173	65.2	153	33.2	118	27.4	51.1	12.9
(SD)	(28.0)	(29.6)	(11.0)	(12.9)	(18.4)	(12.2)	(23.7)	(6.1)

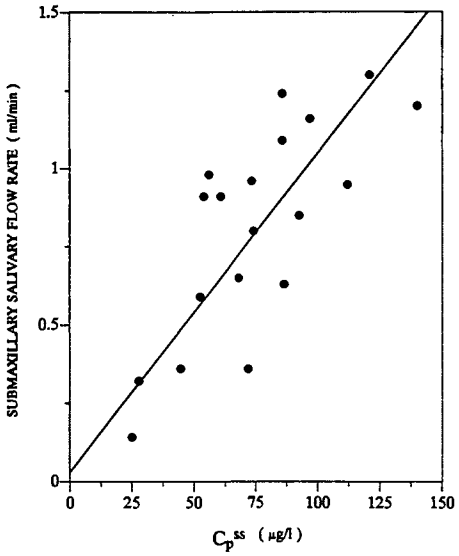

Fig. 2. Steady-state submaxillary salivary flow rate as a function of the steady-state plasma concentration associated with intravenous pilocarpine infusions of 90–240 µg/kg/hr × 2.5 hr.

plasma concentration range of 10–120 µg/L. These levels induce salivary flow rates (parotid + submaxillary) of up to several milliliters per minute without evidence of tachyphylaxis. Modest concentrations of pilocarpine induce salivary flow rates of several microliters per minute with minimal side effects. Once steady state is achieved there is excellent correspondence between plasma concentrations and salivary flow rates. The postinfusion decline in salivary response is log-linear over time, with a half-life approximating the terminal half-life of pilocarpine in plasma, about 1.3 hr.

While one would prefer to perform such studies in conscious animals, anesthesia is necessary. There is little doubt

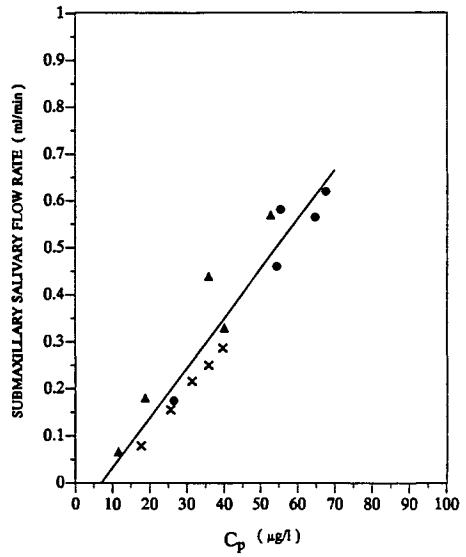

Fig. 3. Postinfusion submaxillary salivary flow rate as a function of plasma concentration associated with three intravenous 2.5-hr infusions of pilocarpine to dog two. Slope = 0.011 µg/min/L-min; intercept = −0.075 ml/min; r = 0.95. (×) 90 µg/kg/hr; (▲) 162 µg/kg/hr; (●) 240 µg/kg/hr.

Table III. Postinfusion Analysis of Submaxillary Saliva Flow Rate as a Function of Plasma Pilocarpine Concentration

Dog	Slope (µg · ml/L · min)	Intercept (ml/min)	r
1	0.012	−0.028	0.92
2	0.011	−0.075	0.95
3	0.016	−0.042	0.97
4	0.018	−0.076*	0.97
5	0.012	−0.081	0.85
6	0.009	−0.066	0.95
Mean	0.013	−0.060	
(SD)	(0.003)	(0.019)	

* Statistically different from zero (P = 0.024).

that the pentobarbital, although unlikely to induce the esterases thought to mediate the metabolism of pilocarpine, may have affected salivary flow through its effects on hemodynamics. It should be noted, however, that the linear relationships observed between concentration and effect in each dog involved composite data from as many as four different experiments and an equal number of anesthetic events. Regardless, there is excellent consistency among the data, supporting the supposition that any effects were consistent among experiments.

Because only a small volume of saliva might be necessary to ameliorate the symptoms of xerostomia, one may speculate that relatively low plasma levels, in the range of 1–10 µg/L, would both behave linearly and induce potentially useful clinical responses. This experimental approach may prove applicable to human studies aimed at determining pharmacokinetic and pharmacodynamic parameters useful for controlling xerostomia.

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