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Scopolamine Sublingual Spray: An Alternative Route of Delivery for the Treatment of Motion Sickness

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ABSTRACT The purpose of this study was to develop a sublingual drug delivery spray formulation of scopolamine hydrobromide (L-(-)-hyoscine hydrobromide) and to determine the absolute bioavailability of scopolamine hydrobromide following sublingual delivery and to investigate the effect of a bioadhesive on the pharmacokinetic parameters of this drug in a rabbit model. Rabbits received a single scopolamine free base equivalent sublingual dose of 100 μg/kg and this was compared to intravenous administration of the drug. Blood samples were collected at different time points, and plasma scopolamine concentrations were determined using a new sensitive and specific LC/MS analytical method which utilized electrospray ionization detection. The bioavailability of sublingual scopolamine was determined by comparing plasma concentrations after sublingual spray delivery with equivalent intravenous doses. Following delivery of the sublingual spray dose, the average C_{max} was 1024.4 ± 177 ng/mL, and the AUC value was found to be 61067.6 ± 9605 ng.min/mL. Relative to the intravenous dose (100% bioavailability), the bioavailability was 79.8% after sublingual spray administration. The addition of 2% chitosan, a bio-adhesive material and an absorption enhancer, showed a significant improvement in scopolamine sublingual absorption ($p < 0.05$) was observed. Considering the limitations of delivering scopolamine orally or transdermally to patients who experience motion sickness, the sublingual route of administration using a spray delivery dosage form, is a potential alternative modality for the prevention of nausea and vomiting associated with motion sickness.

KEYWORDS Scopolamine, Chitosan, Sublingual, Spray, Motion sickness

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INTRODUCTION

Scopolamine [L-(-)-hyoscine] (Fig. 1) has been the drug of choice for the symptomatic treatment of motion sickness for many years (Money, 1970; Holling et al., 1944). This belladonna alkaloid exerts its action by competitively inhibiting the action of acetylcholine at muscarinic receptors and acts as a nonselective muscarinic antagonist, producing peripheral antimuscarinic and

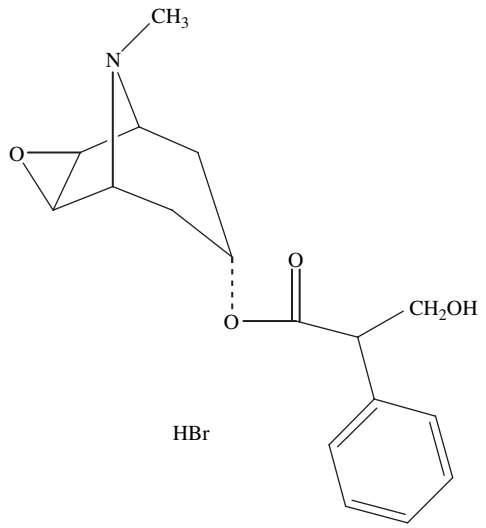

FIGURE 1 **Structure of Scopolamine Hydrobromide [L-(-)-hyoscine hydrobromide].**

central sedative, antiemetic, and amnestic effects (Ali-Melkkila et al., 1993). Currently, four dosage forms for the administration of scopolamine are marketed. These include parenteral injection, ophthalmic solution, oral tablets, and skin patches; the last two dosage forms are used most commonly for prevention of motion sickness. The intravenous route is 100% bioavailable; however, the invasive nature of the IV procedure, and occasional technical constraints, such as those experienced during air flights, limit the usefulness of this route of administration. The variability in absorption and poor bioavailability (10.7–48.2%) after oral administration indicate that this route is neither reliable nor effective for this drug (Putcha et al., 1989). Transdermal administration of scopolamine also has its limitations. For example, plasma concentrations of the drug after transdermal administration indicate major inter-individual variations (Renner et al., 2005). Moreover, the patch released 0.5 mg of alkaloid over a relatively long period of 72 hr, and scopolamine concentrations in plasma also declined more slowly after the patches were removed relative to after an IV dose (Shaw et al., 1980).

While oral or transdermal systems can provide delivery of scopolamine to a person who suffers from motion sickness, there is necessarily a delay in onset of action before the effective entry of scopolamine into the patient circulation. For example, the peak plasma concentration is not reached until 12–16 hr after transdermal dosing (Cintron et al., 1987; Shaw et al., 1980); and it has been reported in normal subject that t_{max} after oral administration is 0.78 ± 0.1 hr (Putcha et al., 1996). Furthermore, in the case of a sudden air or water turbulence, a individuals who have not anticipated the need for a motion sickness medication may suddenly find themselves in a situation where they feel an instant need for such medication. The reported delay in the drug reaching the circulation after patch application, and the potential for prolonged untoward side effects such as dry mouth, dizziness, blurred vision, confusion, and hallucinations, led us to explore the use of a sublingual spray dosage form of scopolamine as an alternative to oral or transdermal delivery especially that previous studies have shown suitability of the nasal mucosa for scopolamine delivery (Putcha et al., 1996; Kloecker et al., 2001).

The sublingual route (physiological pH ~6.5; Quintanar-Guerrero et al., 2001) has the potential for providing an alternative to intravenous dosing for rapid delivery of drugs to the systemic circulation. It was found that only 2.6% of nonmetabolized scopolamine is excreted in the urine, which suggests significant first-pass metabolism after oral administration of scopolamine (Renner et al., 2005). In this respect, sublingual drug delivery by-passes gastrointestinal and hepatic presystemic elimination, and is an acceptable form of drug delivery in patients with swallowing problems. A mucoadhesive addition to the sublingual dose might enhance the contact time with the sublingual mucosa and further enhance drug bioavailability (Surapaneni et al., 2006).

Scopolamine has a weak basic character (pKa = 7.6) and reasonable lipid solubility with a log p value of 0.98 (Li, 2005). Previous studies have shown that the absorption of scopolamine hydrobromide across the nasal mucosa was rapid and had a similar bioavailability to that of an intravenous dose of the drug. It was hypothesized that sublingual administration of the drug could provide a similar effect. The objective of this study was to develop a sublingual spray delivery system of scopolamine and investigate the absolute bioavailability as well as other pharmacokinetic parameters in a rabbit model. The effect of including chitosan, a bio-adhesive material and an absorption enhancer, in the sublingual spray formulation, was also investigated.

MATERIALS AND METHODS

Materials

Scopolamine hydrobromide trihydrate, ethanol, propylene glycol, chlorobutane, mannitol, chitosan,

and sodium phosphate were obtained from Sigma-Aldrich Chemical Co. (St Louis). Hydrochloric acid, purified water USP, ammonium acetate, methanol, and HPLC grade acetonitrile were obtained from Fisher Scientific (Pittsburgh, Pennsylvania). Water for HPLC was passed through a reverse osmosis system (Milli-Q® Reagent Water System) before use. Isoflourane gas for anesthesia was provided by VMC Anesthesia (Ohmeda Waukesha, WI). Siliconized microcentrifuge tubes, vials, and tips were purchased from Fisher Scientific (Fair Lawn, NJ). Saline (0.9%, injectable) was purchased from Baxter Healthcare Corporation (Deerfield, IL). Heparinized caraway capillary tubes were purchased from Baxter Healthcare Corporation (McGraw Park, IL). Tuberculin Slip tip Sterile Catheters were purchased from J&J Medical (New Brunswick, NJ).

Animals

Male New Zealand albino rabbits weighing between 3.0–3.5 kg (Myrtle's Rabbitry Inc., Thompson Station, TN) were used. The animal work was conducted at the University of Kentucky Chandler Medical Center, Division of Laboratory Animal Resources (DLAR). All research and testing activities related to this work were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) prior to the initiation of this research, and during its execution.

In Vivo Sublingual and Intravenous Studies

Following introduction of anesthesia (isoflourane general anesthetic gas which was introduced from a cylinder through a nose mask) a catheter was placed in the marginal ear vein of the rabbit for blood sample collection. Rabbits were under anesthesia for at least half the experimental time. For sublingual spray administration, a scopolamine dose of 100 μg/kg (0.1 mL) in either formulation solution SL or SL-CH (Table 1) was applied to the sublingual mucosa of the rabbit utilizing a spray bottle comprising a sealed container fitted with an actuator, and a metering chamber in communication with the formulation by means of a dip-tube ($n = 3$ rabbits/formulation per route). An initial separate in vitro spray weight evaluation was performed with the spray bottle prior to animal dosing. The spray bottle was hand-filled with 2.5 mL of deionized water and actuated 10 times for priming before obtaining spray weight data. After priming, net spray weight measurements were taken for 10 consecutive actuations. Target delivery weight for each single spray actuation was 0.1 ± 0.01 g. For intravenous administration, scopolamine hydrobromide aqueous solution was utilized; a sterile drug solution was prepared by filtration (double 0.22 μm filters), and a dose of 100 μg/kg scopolamine was injected into the marginal ear vein cannula followed by a 0.1 mL flush with 10% (v/v) heparin/normal saline solution to keep the cannula patent.

TABLE 1 **Active and Inactive Constituents of Scopolamine Hydrobromide Sublingual Spray Formulation Solutions SL and SL-CH**

Material	Formulation SL	Formulation SL-CH
Scopolamine HBr.3H_2O	433.5 mg	433.5 mg
Absolute alcohol	30 mL	30 mL
Mannitol	400 μg	400 μg
Propylene glycol	5 mL	5 mL
Chitosan	–	2 mg
Phosphate buffer (0.5 M, pH 3.5)	100.0 qs*	100.0 qs*

*qs indicates addition of a sufficient quantity to afford a final volume of 100 mL.

Blood samples (1 mL) were collected as follows at the following times: at baseline, before scopolamine hydrobromide dose administration; immediately after scopolamine hydrobromide administration; and subsequently at 5, 10, 20, 45, 60, and 120 min following scopolamine hydrobromide administration. Blood samples were injected into preheparinized tubes and immediately placed on ice. Plasma was separated by centrifugation at 3000 rpm for 10 min, placed in polypropylene tubes, and frozen at –20°C until the time of analysis.

Sample Preparation

Chlorobutane (1 mL) was added to 500 μL of each plasma sample in 2 mL polypropylene test tubes. The samples were vortexed for 60 sec and centrifuged at 8,000rpm for 10 min. An aliquot part (800 μL) of the resulting supernatant was directly transferred to autosampler vials, evaporated to dryness with nitrogen gas at ambient temperature, and then reconstituted with 100 μL methanol. Aliquot parts (25 μL) of this final solution were then injected onto the HPLC-MS system.

HPLC-MS Analysis

Chromatography was performed on a Waters Sunfire C_{18} (4.6 × 250 mm, 5 μm) column with a mobile phase consisting of 30% ammonium acetate (10 mM, pH 4), 40% methanol, and 30% acetonitrile. The flow-rate was set at 0.3 mL/min. The LC-MS system consisted of a Waters 2690 HPLC pump (Waters, Milford, MA), a Waters 2695 autosampler, and a Micromass ZQ detector (Waters, Milford, MA) which utilized electrospray ionization (ESI) detection. Selected ion monitoring (SIM) was performed in the positive mode for scopolamine, M+ = 304 m/z (dwell time 0.8 s), the capillary voltage was 3.30 kV and the cone voltage was 32 V. The source block and desolvation temperatures were 100 and 300°C, respectively. Nitrogen was used as the nebulization and drying gas at flow rates of 70 and 450 L/hr, respectively. Calibration curves were constructed using a linear regression of the drug peak area versus nominal drug concentrations. The method was validated over the concentration range used, and found to be satisfactory for the determination of scopolamine in rabbit plasma over the concentration range 10–2000 ng/mL. The limit of quantification (LOQ) was established at 10 ng/mL. MS control and spectral processing were performed using MassLynxTM software, version 3.5 (Waters).

Pharmacokinetic Analysis

Concentration-time profiles of scopolamine after IV and sublingual administration of formulation solution SL or SL-CH were evaluated by a non-compartmental model (WinNonlin Professional, version 4.1, Pharsight Corporation, Mountain view, CA). Pharmacokinetic parameters, such as terminal elimination half life ($t_{1/2}$), area under the curve from 0 to infinity, $AUC_{0-\infty}$ were estimated using this software.

After a single dose, maximum plasma concentration (C_{max}), and time to reach maximum concentration (T_{max}) were also determined. The absolute bioavailability of the sublingual formulation was calculated from Eq. (1):

$$F = \frac{AUC_{SL}}{AUC_{IV}} \times \frac{Dose_{IV}}{Dose_{SL}} \times 100 \qquad (1)$$

Where F is the percent absolute bioavailability, and AUC_{SL}, AUC_{IV}, $Dose_{IV}$, $Dose_{SL}$ are the area under the curve for the sublingual and intravenous administrations and corresponding dose for these routes of administration, respectively. Data were evaluated using two-tailed *t*-test statistical analysis.

RESULTS AND DISCUSSION

Fig. 2 illustrates the mean plasma concentration *versus* time curves for scopolamine obtained after IV injection and sublingual spray administration of formulation SL at a dose of 100 μg/kg in the rabbit. Following IV injection, the disposition of scopolamine was described by a semi-log curve, with a terminal elimination half-life of 34 ± 3.91 min.

The concentration-time profiles were analyzed by a non-compartmental method, and pharmacokinetic parameters were determined. The mean AUC values for scopolamine after IV and sublingual spray delivery of formulation SL were 76512.8 ± 10273 ng.min/mL, and 61067.6 ± 9605 ng.min/mL, respectively (Table 2). The absorption enhancer chitosan was used in formulation SL-CH; this formulation increased the AUC to 70042.2 ± 11125 ng.min/mL. Chitosan is a natural linear cationic polysaccharide obtained by deacetylation of chitin. Chitin is the second most abundant polysaccharide in nature after cellulose. The main commercial sources of chitin are the shell wastes of shrimp, crab, lobster, krill, and squid. Chitin is considered to be a biologically safe, nontoxic, biocompatible, and biodegradable polysaccharide (Patel et al., 2005).

Following sublingual spray administration, the bioavailabilities of formulation SL and formulation SL-CH were found to be 79.8 ± 1.9% and 91.5 ± 2.3%, respectively (Fig. 3). Scopolamine sublingual bioavailability can be improved by coadministration of a

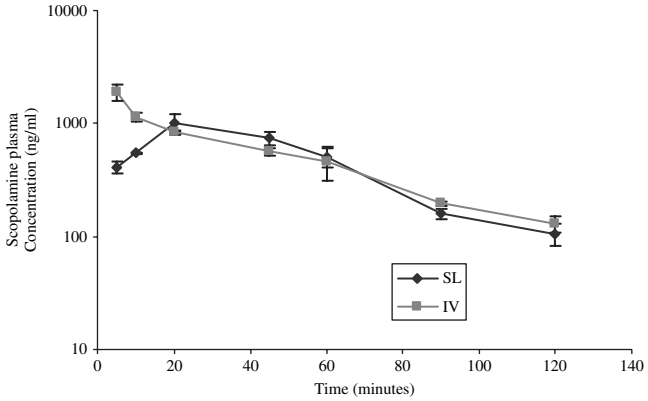

FIGURE 2 **Mean Plasma Scopolamine Concentration Versus Time Curves After Sublingual Spray Formulation SL and Intravenous Administration at 100 μg/kg Scopolamine Equivalent Dose (n = 3). All Values Show the Mean ± SEM.**

TABLE 2 **Area Under the Curve and Absolute Bioavailability of Scopolamine Sublingual Spray Formulation SL and Formulation SL-CH in Rabbits (n = 3)**

Dose 100 mg/kg scopolamine equivalent	Route	AUC_{∞}(ng.min/mL) mean ± SE	Absolute bioavailability (%) mean ± SE
	Intravenous	76512.8 ± 10273	100
	Sublingual	–	–
	Formulation SL	61067.6 ± 9605	79.8 ± 1.9%
	Formulation SL-CH	70042.2 ± 11125	91.5 ± 2.3%

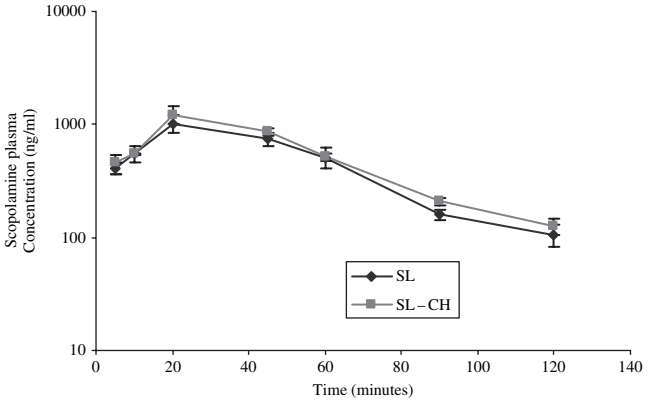

FIGURE 3 **Mean Plasma Scopolamine Concentration Versus Time Curves After Sublingual Spray Dosing (100 μg/kg Scopolamine Equivalent Dose) to Rabbits Without (Formulation SL) and With (Formulation SL-CH) Chitosan (n = 3). All Values Show the Mean ± SEM.**

mucoadhesive and an absorption enhancer such as chitosan. While the enhancers significantly improve drug uptake, they exert considerable membrane damaging effects as evidenced by protein release and histological examinations (Shao et al., 1992). Sublingual formulations containing 2% chitosan proved to be safe with enhanced mucoadhesion and drug release properties (Varshosaz et al., 2006).

The addition of 2% chitosan in the formulation significantly improved scopolamine bioavailability and the extent of sublingual absorption. This improvement in bioavailability may be due to one or more factors, such as an increase in the mucosal lipid fluidity, or direct loosening up of the tight junctions of the epithelia (Chun-Ying et al., 2005). Tight junctions are the cell-to-cell junctions that seal adjacent epithelial cells together; they are caused by the attachment of the actin filament system from one cell to that of a neighboring cell, preventing the passage of most dissolved molecules from one side of the epithelial sheet to the other (Asada et al., 2003). Furthermore, chitosan acts as a muco-adhesive material by binding strongly to negatively-charged biological surfaces such as mucous membranes (Pavis et al., 2002). Two-tailed t-test statistical analysis indicated that there was a significant difference in scopolamine sublingual bioavailability between in formulations with and without the penetration enhancer chitosan ($p < 0.05$).

The pharmacokinetic parameters of the two sublingual formulations, together with the IV pharmacokinetics are presented in (Table 3). The terminal half-life of scopolamine after intravenous administration was close to the terminal half-life after sublingual administration. This result implies that there is no long-term deposition of scopolamine in the sublingual mucosa after transmucosal delivery. Furthermore, the penetration enhancer chitosan increased the C_{max} from 1024.4 ± 177 to 1221.7 ± 216 ng/mL, but the time to reach that concentration (t_{max}) was measured at 20 min after spray solution application of either of the formulations to the rabbit sublingual mucosa.

The short sublingual t_{max} value, which is close to that obtained after nasal administration, is an important criterion for a fast onset of action in the treatment of motion sickness.

The above data indicate that a sublingual spray formulation of scopolamine results in a rapid onset of action. Furthermore, sublingual formulations containing 2% chitosan as a mucoadhesive and penetration enhancer, affords scopolamine bioavailability close to that obtained from an IV dose (i.e., ~90%), which is

TABLE 3 **Pharmacokinetic parameters following sublingual spray administration of formulation SL and formulation SL-CH in rabbits (n = 3)**

Parameter	Formulation SL	Formulation SL-CH	Intravenous
C_{max} (ng/mL)	1024.4 ± 177	1221.7 ± 216	1894.1 ± 454.9
t_{max} (min)	20 ± 0.91	20 ± 0.86	0
$t_{1/2}$ (min)	27.9 ± 2.33	28.7 ± 2.96	34 ± 3.91

expected, since the sublingual dose escapes first pass metabolism by the liver and the gastrointestinal tract.

CONCLUSION

Considering the limitations of delivering scopolamine orally or transdermally to patients who suffer from motion sickness, delivery of this drug by the sublingual route, utilizing a spray delivery dosage form formulation, is a potential alternative modality for the prevention of nausea and vomiting associated with motion sickness. When given sublingually, scopolamine is well absorbed and its bioavailability by this route is significantly enhanced with the addition of the bio-adhesive material and permeation enhancer chitosan to the sublingual spray formulation. Further studies will be needed to evaluate the clinical potential of this route of delivery for scopolamine.

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