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Pharmacokinetics and Safety of Sublingual Flumazenil (CRLS035) in Healthy Adults (Potential Therapy for Hepatic Encephalopathy)

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Abstract

Flumazenil, a $GABA_A$ receptor antagonist, has a significant clinical benefit especially in overt hepatic encephalopathy patients, although it requires intravenous access. A novel highly concentrated sublingual spray formulation of flumazenil (CRLS035) was developed by Coeruleus Ltd. The aim of this study was to determine the single dose safety and pharmacokinetics of sublingual CRLS035 versus flumazenil intravenously (IV) in healthy volunteers.

Ten healthy adult volunteers participated in the study. CRLS035 was administered sublingually in two doses (1.1 mg and 2.2 mg) vs. IV flumazenil (0.2 mg). Subjects were evaluated after a high-fat diet and water consumption. Blood samples were collected pre- and post-dose at eight time points. Flumazenil levels were analyzed for Cmax, Tmax, Cmin, Tmin, AUC0-∞, AUC0-t and T1/2. Safety variables included local oral area and assessment of systemic adverse events.

The estimated bioavailability of the two sublingual doses was 14% and 11%, respectively. The bioequivalence of the 1.1 mg sublingual dose was similar to the 0.2 mg IV dose. Water consumption and the high-fat diet did not change the pharmacokinetic parameters significantly. No associated adverse events were reported across the study.

The pharmacokinetics of sublingual flumazenil is comparable to intravenous administration and the drug is safe. The sublingual approach allows convenient and better treatment availability for patients with hepatic encephalopathy.

Keywords: Sublingual flumazenil; CRLS035; Hepatic encephalopathy; Insomnia

Introduction

Hepatic encephalopathy (HE) is a complex neuropsychiatric disorder secondary to acute or chronic liver failure [1,2]. Although the exact pathophysiology of HE has not been clarified, enhanced central nervous system inhibition at the γ-aminobutyric acid (GABA)-benzodiazepine receptor complex, mediated by increased levels of endogenous benzodiazepine-receptor ligands (BZRL), has been proposed [1-4]. Flumazenil is a $GABA_A$ receptor antagonist [5], able to reverse the hypnotic effect of 90% of the sleep-related hypnotics [1]. In addition, a subset of HE patients who express high levels of benzodiazepine-like compounds in their blood showed a clinical benefit from this therapy [6]. Experience with this drug has been gained mainly in patients in acute states, and there is minimal experience in patients with chronic HE [6,7].

The efficacy of flumazenil was evaluated in a large study, including 527 patients with grade III and IVa HE patients. Those assigned to the treatment group received flumazenil 1 mg IV, in addition to lactulose 30 mL every 6 hours. Significant improvements were seen in the treated group in neurologic scores after three hours. Of the patients with grade III HE at baseline, 17.5% demonstrated improved neurologic scores as compared with 3.8% in the placebo group. The corresponding numbers among the patients with grade IVa HE were 14.7% versus 2.7%, respectively [6,8].

Flumazenil has been in wide use for over 20 years as a benzodiazepine antidote, often used after surgical procedures to accelerate the arousal of patients after benzodiazepines use [9].

Due to the fact that the drug is available only as an intravenous drug, we believe that its use is still limited. Coeruleus Ltd. has developed a novel highly concentrated sublingual flumazenil spray (CRLS035). This product is anticipated to significantly improve the functionality and quality of life for those who suffer from acute and chronic hepatic encephalopathy, and after the use of benzodiazepines in anaesthetic settings.

Previous study examined the safety and efficacy of sublingual flumazenil in reversing the residual hypnotic effect of zolpidem and brotizolam in 20 healthy subjects [9]. Flumazenil was superior to placebo by 59% to 93% (P<0.05-0.001) and subjects reported significant improvement in vigilance with flumazenil, both at 20 min and 60 min, as was also seen in cognitive studies [9].

The aims of the current study were to determine the pharmacokinetic (PK) profile and the safety of a single administration (two doses) of CRLS035 in 10 healthy subjects. The results of this study were collected, reported and verified according to GCP guidelines, the company SOPs and the local authorities guidelines.

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CRL0033 – 1.1% Flumazenil (%w/w)	
Flumazenil	1.1
Ethanol absolute	40.0
Propylene glycol	10.0
Citric acid anhydrous	0.05
Sodium citrate dehydrate	0.05
Nicotinamide	1.5
L-menthol	0.1
Water for injection	47.0

Table 1: CRLS035 formulation preparation.

Patients and Methods

The study was conducted at Rambam Health Care Campus in Haifa, Israel, from June 29, 2012 (first screening day), until August 21, 2012 (last follow-up phone call day).

The study was designed as an open label, randomized, three-way crossover. Pharmacokinetics were analysed using the marketed IV flumazenil formulation as the comparator.

The research protocol and Informed Consent Documents were reviewed and approved by the Institutional Review Board (IRB) of the clinical site. Participants were compensated for time and expenses**.**

Study drug

Flumazenil for sublingual administration is a transparent solution containing 11 mg/ml flumazenil and excipients (Table 1). CRLS035 formulation development was performed for Coeruleus by Nextar ChemPharma Solution Ltd. The desired formulation was obtained following a series of formulation optimizations with several excipients and based upon their mucosal absorption, solubility and initial stability.

5 ml Type I glass vials (Saint Gobain) with pumps (Pump 100 µl Pfeiffer) delivering 0.1 ml (metered dose-1.1 mg) per puff were used. Pumps were routinely tested for accuracy and reproducibility by the manufacturer. All the excipients are well known and used frequently as oral/sublingual medication. Due to the low intended flumazenil administration volume (0.1-0.2 ml per day), the amount of each excipient in the formulation are far below the maximal FDA approved daily dosages.

The study was designed for 1.1 mg and/or 2.2 mg (1 or 2 puffs). The IV dose was 0.2 mg. Standard IV flumazenil was provided to the study from the hospital-site pharmacy.

Participants

Ten healthy volunteer subjects, four women and six men, aged ≥18, attended five visits, a screening visit and four treatment visits. The 10 subjects who fulfilled all the following criteria were included in the study: the subject signed an informed consent form prior to any study-mandated procedure, male or female aged ≥18 at screening, women of childbearing potential must have a negative pregnancy test at the screening visit and use a reliable method of contraception during the entire study duration (e.g. contraceptive pill; intra-uterine device; contraceptive injection (prolonged-release gestagen); subdermal implantation; vaginal ring or transdermal patch), body mass index ≥18.5 and <32 kg/m^2, in good health as determined by medical history, physical examination and ECG, and no history of use of illicit drug, alcohol (ethanol) or stimulants.

Any of the following was regarded as a criterion for exclusion from the trial: any use of medications within one month prior to the screening visit, except for contraceptive pills, previous exposure to benzodiazepines and/or non-benzodiazepine hypnotic drugs within three months prior to study initiation, history of epilepsy and or anti-epileptic drugs, pregnancy or breast feeding, clinically relevant ECG abnormalities, history of alcohol or drug abuse within three years prior to the screening visit, known hypersensitivity to drugs of the same class as the study treatment, or any excipients of the drug formulation, treatment with another investigational drug within one month prior to the screening visit, history of severe head injury, any acute or chronic illness or xerostomia (endogenic or drug induced).

Study design

Subjects who signed informed consents were invited for a screening visit within 14 days. At the end of the screening process, the medical practitioner decided on the eligibility of the subject. If the subject was eligible for the study, he/she was assigned to study arm A or B. Female participants were administered a urine pregnancy test (human chorionic gonadotropin (HCG)) at the screening visit and immediately prior to each experimental session.

Treatment visits were performed on 7 ± 2 days. Each subject was treated according to his study arm assignment (Table 2). Blood samples for flumazenil levels were taken at T=0 (pre-dosing), 10 min, 30 min, 60 min, 90 min, 2 h, 4 h, 6 h, and 12 h.

All treatment visits started at 7:00 am, after 10 hours of fasting.

Drug administration

At time 0, sublingual CRLS035 or IV flumazenil (0.2 mg) was given over 15 seconds. Flumazenil administration (SL or IV) was given with subjects lying in a hospital bed. Subjects were not allowed to leave the bed or to eat for four hours. Water was allowed one hour post-dose. Standard food was served at t=4 h; t=7; t=10; t=13.

The following concomitant therapy or medications were prohibited: any use of medication except contraceptive pills, smoking during a study visit, consumption of alcohol or grapefruit (including as juice) from the day prior to each of the study visits and for three consecutive days.

The following concomitant therapy or medication were allowed: any diet or non-pharmacological activity was allowed if started at least one month prior to the screening visit and remained stable until 24 hours after the last administration of study treatment.

At the first 4 h post-dosing, the subjects were lying in bed. Water was allowed one hour post-dose. Standard food was allowed four hours post-dose. Afterward, subjects were allowed to resume regular activities with no unusual efforts. In visits where water or food consumption effects were evaluated, water or food was allowed according to the protocol.

Blood sampling for flumazenil and for lab test, full physical examination including the sublingual and oral areas, concomitant medication inquiry, vital signs measurements, ECG, and adverse events

Group Assignment	Week 1 Visit 2	Week 2 Visit 3	Week 3 Visit 4	Week 4 Visit 5
Sequence A N=5	S/L 1.1 mg	S/L 2.2 mg	IV 0.2 mg	S/L 2.2 mg with 240 ml water
Sequence B N=5	IV 0.2 mg	S/L 2.2 mg	S/L 1.1 mg	S/L 2.2 mg with high fat diet

Notes: 0.2 mg flumazenil IV was given over 15 seconds; S/L 1.1 mg is equal to one puff of CRLS035; S/L 2.2 mg is equal to two puffs of CRLS035

Table 2: Treatment groups.

(AE) inquiry were performed according to the protocol. At the end of the visit, subjects were provided with instructions for the next visit.

For subjects in study Arm A visit 5, water consumption effect: 240 ml of water was administered 10 min post-dosing.

For subjects in study Arm B visit 5, high-fat diet effect: high-fat diet was administered 30 min before the drug. Subjects ate the meal in 30 min or less.

A high-fat (approximately 50% of total caloric content) meal included approximately 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively.

Subjects were contacted by telephone seven days after the last treatment visit and were asked to report if any AE had occurred since their last visit.

Adaptive dose selection and administration

According to treatment group and visit (Table 2), subjects received the drug either sublingually in two doses (100 or 200 µl; 1.1 mg and 2.2 mg, accordingly) or with IV flumazenil (0.2 mg). In addition, subjects were evaluated after the high-fat diet and water consumption.

Flumazenil bioanalysis

Flumazenil plasma concentrations were determined by liquid chromatography with double mass spectrometry detection (LC-MS/MS). The lower calibration range was from 0.1-50 ng/mL and the higher calibration range was from 0.5-100 ng/mL, with the lower limit of quantitation set at 0.1 ng/mL. The internal standard was zaleplon-d_5, N-[3-(3-cyanopyrazolo-[1,5-α]pyrimidin-7-yl)phenyl]-N-d_5-ethyl-acetamide.

Values reported as below the limit of quantitation, 0.1 ng/mL, were set at 0 ng/mL. Non-compartmental pharmacokinetic parameters and statistics were calculated using Phoenix WinNonlin 6.3. Excel 2010 was used for data collection and to prepare the graphs. The maximum concentration, C_{max}, and time of maximum concentration, T_{max}, were determined as the maximum measured concentration and its associated time. The area under the plasma concentration curve from 0 h to the last measurable concentration, AUC_{0-t}, was calculated using trapezoidal estimation, and $AUC_{0-\infty}$ was extrapolated from AUC_{0-t} using the terminal rate constant. Values for half-life, $t_{1/2}$, were calculated using the last three to five non-zero values and were considered reliable if the coefficient of determination, r^2, was >0.8. The values for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were normalized by dose (NC_{max}, $NAUC_{0-t}$, and $NAUC_{0-\infty}$).

Safety measures

Based on flumazenil's generic nature, broad clinical experience using systemic exposure and wide safety margins (of up to 3 mg flumazenil IV per patient and up to 600 mg per oral delivery), the tested dosages in this study were safe.

In this study, the sublingual route was tested, so local safety was assessed in addition to overall safety. Safety assessments were collected using AE inquires, measurements of the safety variables and subjects' reports.

CBC, full biochemistry and liver function tests (SMAC) as well as oral and mucosal area examination were performed at the screening visit and at all treatment visits (time-points according to protocol).

AEs were reported and graded by the investigator throughout the study. No SAEs occurred in this study. Adverse events inquiry was performed by site staff at T=6 h, T=12 h and one week post-treatment. Subjects were asked to contact the site in the event of any AE.

Assessment of efficacy

In this study, the PK profile of sublingual administration of CRLS035 was determined in comparison to the intravenous standard dose of 0.2 mg. Blood samples were collected at nine time-points (pre-dose, T=10 min, T=30 min, T=60 min, T=90 min, T=2 h, T=4 h, T=6 h and T=12 h) for further measures of flumazenil levels using HPLC-MS/MS.

Statistical analysis

The study was designed and conducted as an open label, randomized, three-way crossover study. PK parameters were to be compared to the IV administration. C_{max}, T_{max}, C_{min}, T_{min}, $AUC_{0-\infty}$, AUC_{0-t}, $T_{1/2}$, and F would be calculated. ANOVA was planned to be used for comparison of all PK measures (sublingual, IV, high-fat and water consumption).

For safety analysis, summary data would be presented for the overall population. For continuous variables, descriptive statistics (n, mean, standard deviation, median, minimum and maximum) would be provided for actual values and changes from baseline, as appropriate.

The determination of sample size was based on statistical considerations. Based on a STD of 1.5, alpha value of 0.05, and a desired power of 80%, the sample size was 5 subjects/group. It was planned to enroll 10 subjects.

	Flumazenil Concentrations (ng/mL)									
	Sublingual, 1.1 mg Fasting, no water n = 10		Sublingual, 2.2 mg Fasting, no water n = 10		Intravenous, 0.2 mg n = 10		Sublingual, 2.2 mg 240 mL water n = 5		Sublingual, 2.2 mg High fat meal n = 5	
Time (hr)	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
0	0		0		0		0		0	
0.167	4.55	± 1.61	6.00	± 3.05	45.9	± 63.9	7.26	± 3.15	5.26	± 2.43
0.5	4.75	± 1.11	5.57	± 1.82	3.44	± 3.41	6.66	± 1.79	4.00	± 1.49
1	3.16	± 0.79	5.09	± 1.59	1.51	± 1.01	4.38	± 1.09	3.20	± 1.41
1.5	2.21	± 0.96	3.32	± 1.15	1.01	± 1.15	2.91	± 0.66	2.39	± 1.11
2	1.37	± 0.54	2.23	± 0.68	0.58	± 0.38	1.96	± 0.26	1.91	± 0.75
4	0.48	± 0.21	0.65	± 0.30	0.26	± 0.14	0.41	± 0.18	0.47	± 0.13
6	0.02	± 0.06	0.08	± 0.14	0		0		0.02	± 0.05
12	0		0		0.03	± 0.09	0		0.04	± 0.08

Standard deviations were not calculated when all values were 0 ng/mL.

Table 3: Mean Plasma Concentrations of Flumazenil.

Results

Study subjects

All 10 subjects who started the study attended all visits. Subjects ranged in age from 20.7 years to 28 years (mean, 23.9 years). Weight ranged from 53 kg to 81 kg (mean, 72 kg), and height ranged from 163 cm to 185 cm (mean, 174 cm), with normal BMI indices. All subjects were white (Arabs and Jews living in Israel). None of the 10 subjects used any medications during the study (except for one female subject who used contraceptive pills before and during the study). Eight subjects were non-smokers, one was a past smoker and one was a smoker. All pregnancy tests were negative.

Plasma concentrations of flumazenil

The mean plasma concentrations and standard deviations for each of the treatments are shown in table 3. Figure 1 is a semi-log plot of the mean concentrations for 1.1 and 2.2 mg CRLS035 sublingually administered, or 0.2 mg flumazenil intravenously administered to fasting subjects. Figure 2 is a semi-log plot of the mean concentrations for 2.2 mg CRLS035 administered sublingually to fasting subjects without any water or food, to fasting subjects with subsequent administration of 240 mL water, and to subjects 30 min after a high-fat meal.

For each of the sampling times, the mean concentrations of flumazenil were higher after administration of the 2.2 mg sublingual flumazenil to fasted subjects than after administration of 1.1 mg sublingual flumazenil. Intravenous administration of the much lower 0.2 mg dose produced a much higher mean concentration at 10 min post-dose than the concentrations observed at the same time for 1.1 and 2.2 mg sublingually. However, at subsequent sampling times, the concentrations for 0.2 mg IV were lower than for 1.1 and 2.2 sublingually, with the exception of 12 hours post-dose where the level was zero (Table 3, Figure 1). For all sublingual administrations to fasted subjects, the T_{max} values occurred at 0.167, 0.5, or 1 h (data not shown), indicating rapid absorption after sublingual administration. Further, the concentrations at 0.167 h are close to or equal to the C_{max} concentrations, indicating significant concentrations are present at 10 min after sublingual administration.

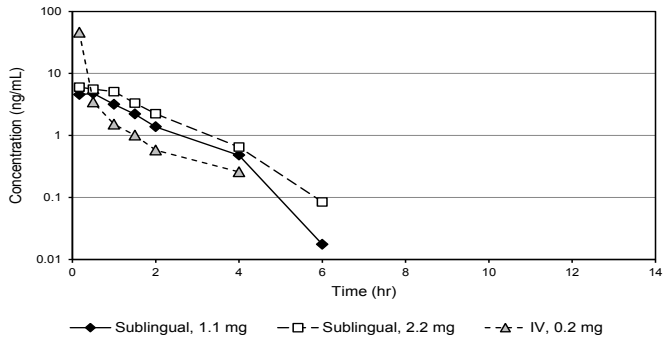

Figure 1: Mean concentrations for sublingual fasting and intravenous (semi-log plot).

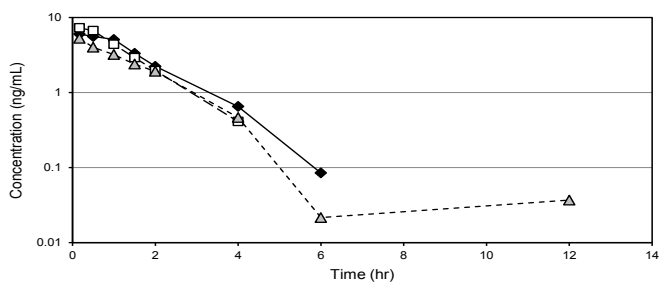

Figure 2: Mean concentrations of 2.2 mg sublingual fasting, water, high-fat meal (semi-log plot).

For the 2.2 mg administered under three conditions, the mean initial concentrations at the first few sampling times were lowest for CRLS035 administered 30 min after a high-fat meal, second lowest for administration to fasted subjects, and highest for administration followed by 240 mL water. At the later sampling times, the mean concentrations were similar for the three conditions (Table 3, Figure 2).

Pharmacokinetic parameters

Table 4 shows the summery statistics for the pharmacokinetic parameters. Table 5 shows the ratios of NC_{max} and $NAUC_{0-t}$ for the individual subjects. Table 6 shows pharmacokinetics parameters of administered to fasted subjects without water, with 240 ml water or with high fat meal. The inter-subject variability for the pharmacokinetic parameters was much higher for intravenous administration than for sublingual administration. The %CV values for C_{max} and AUC_{0-t} were greater than 100% for intravenous administration, and less than 50% for sublingual administration (Table 4). This suggests a possible carry-over from the intravenous formulation to the blood sample due to the use of the same arm for injection and sample collection. Analysis of pharmacokinetics parameters of each subject revealed that a carry over effect may have occurred for 3 subjects. Because of these possible anomalies in the plasma profiles for intravenous administration, the median values are probably the most relevant for comparison between treatments.

The C_{max} and AUC_{0-t} values increased as the dose increased from 1.1 to 2.2 mg for CRLS035 administered sublingually to fasted subjects; however, the increases were less than dose-proportional (Table 4).

The median values for the ratios of NC_{max} for 1.1 and 2.2 mg CRLS035 compared to 0.2 mg intravenously were 0.043 and 0.030, respectively. The median values for the ratios of $NAUC_{0-t}$ for 1.1 and 2.2 mg CRLS035 compared to 0.2 mg intravenously were 0.139 and 0.112, respectively (Table 5). These values indicate that the estimated bioavailability of a 1.1 mg sublingual dose was 14% and the estimated bioavailability of a 2.2 mg sublingual dose was 11%. Although the absolute bioavailability of the sublingual CRLS035 is low (<15%), the exposure is similar for 1.1 and 2.2 mg CRLS035 administered sublingually and 0.2 mg flumazenil administered intravenously, since the AUC_{0-t} values are similar and the plasma concentrations are similar from 0.5 hours onward (Table 4).

The pharmacokinetic parameters were similar for 2.2 mg CRLS035 administered to fasted subjects without water and to fasted subjects with 240 mL water taken 10 min after the drug (Table 6). Administering 2.2 mg CRLS035 30 min after a high-fat meal reduced the mean, geometric mean, and median values for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ by approximately 15-40% compared to administration to fasted subjects but the meal also decreased the time to C_{max}; however, none of the differences were statistically significant (Table 6).

Adverse events

Ten healthy subjects participated in this study. There were no serious adverse or deaths events in this study. Safety evaluation in this study included local safety of sublingual and oral areas, and overall

Parameter	Dose, Route	State	Mean	SD	%CV	Geometric mean	Median	Minimum	Max	N
C_{max} (ng/mL)	1.1 mg sublingual	fasted	5.15	1.46	28.4	4.95	5.12	2.89	7.24	10
	2.2 mg sublingual	fasted	6.98	2.51	36.0	6.61	6.28	4.42	12.2	10
	0.2 mg IV	fasted	45.9	63.8	139	19.9	21.0	3.53	200	10
	2.2 mg sublingual	240 mL water	7.48	2.91	39.0	7.03	6.48	4.10	11.7	5
	2.2 mg sublingual	high-fat meal	5.28	2.41	45.6	4.80	5.36	2.44	8.16	5
T_{max} (hr)	1.1 mg sublingual	fasted	0.433	0.140	32.4	0.402	0.500	0.167	0.500	10
	2.2 mg sublingual	fasted	0.484	0.380	78.7	0.356	0.334	0.167	1.00	10
	0.2 mg IV	fasted	0.250	0.263	105	0.200	0.167	0.167	1.00	10
	2.2 mg sublingual	240 mL water	0.433	0.149	34.4	0.402	0.500	0.167	0.500	5
	2.2 mg sublingual	high-fat meal	0.234	0.149	63.8	0.208	0.167	0.167	0.500	5
C_{last} (ng/mL)	1.1 mg sublingual	fasted	0.417	0.196	47.1	0.368	0.455	0.150	0.660	10
	2.2 mg sublingual	fasted	0.389	0.235	60.5	0.319	0.420	0.100	0.800	10
	0.2 mg IV	fasted	0.295	0.096	32.6	0.281	0.270	0.160	0.460	10
	2.2 mg sublingual	240 mL water	0.410	0.184	44.9	0.378	0.410	0.230	0.680	5
	2.2 mg sublingual	high-fat meal	0.302	0.156	51.8	0.264	0.350	0.110	0.500	5
T_{last} (hr)	1.1 mg sublingual	fasted	4.20	0.63	15.1	4.17	4.00	4.00	6.00	10
	2.2 mg sublingual	fasted	4.80	1.03	21.5	4.70	4.00	4.00	6.00	10
	0.2 mg IV	fasted	4.60	2.67	58.2	4.17	4.00	2.00	12.0	10
	2.2 mg sublingual	240 mL water	4.00	0	0	4.00	4.00	4.00	4.00	5
	2.2 mg sublingual	high-fat meal	6.00	3.46	57.7	5.40	4.00	4.00	12.0	5
AUC_{0-t} (ng•hr/mL)	1.1 mg sublingual	fasted	7.84	2.28	29.1	7.56	7.32	4.61	12.5	7.84
	2.2 mg sublingual	fasted	11.4	3.2	27.8	11.0	10.8	6.41	15.8	11.4
	0.2 mg IV	fasted	15.2	17.0	112	9.53	9.43	1.84	57.6	15.2
	2.2 mg sublingual	240 mL water	10.6	2.5	23.3	10.3	10.0	7.36	13.8	10.6
	2.2 mg sublingual	high-fat meal	8.59	3.60	41.9	8.04	6.47	5.54	13.8	8.59
AUC_{inf} (ng•hr/mL)	1.1 mg sublingual	fasted	8.60	2.20	25.6	8.35	8.32	4.99	12.7	8.60
	2.2 mg sublingual	fasted	12.0	3.2	27.0	11.6	11.3	6.65	16.0	12.0
	0.2 mg IV	fasted	12.9	10.2	78.9	9.54	9.91	2.17	30.3	12.9
	2.2 mg sublingual	240 mL water	11.1	2.6	23.0	10.9	10.3	7.98	14.8	11.1
	2.2 mg sublingual	high-fat meal	9.10	3.31	36.4	8.67	7.04	6.57	14.0	9.10
$t_{1/2}$ (hr)	1.1 mg sublingual	fasted	1.15	0.34	29.6	1.11	1.15	0.687	1.92	10
	2.2 mg sublingual	fasted	1.05	0.25	23.8	1.03	1.03	0.781	1.46	10
	0.2 mg IV	fasted	1.18	0.46	39.2	1.11	1.03	0.802	2.03	6
	2.2 mg sublingual	240 mL water	0.891	0.187	21.0	0.874	1.02	0.660	1.04	5
	2.2 mg sublingual	high-fat meal	1.12	0.19	17.0	1.10	1.07	0.967	1.43	5

Table 4: Summary of statistics for pharmacokinetic parameters.

	NC_{max} (ng/L/mg)			$NAUC_{0-t}$ (ng•hr/mL/mg)			Ratio for NC_{max}		Ratio for $NAUC_{0-t}$ (also F)	
Subject ID	**0.2 mg IV**	**1.1 mg SL**	**2.2 mg SL**	**0.2 mg IV**	**1.1 mg SL**	**2.2 mg SL**	**1.1 SL/ IV**	**2.2 SL/ IV**	**1.1 SL/ IV**	**2.2 SL/ IV**
A01	29.7	5.11	2.74	14.8	9.40	4.92	0.172	0.092	0.635	0.332
A02	18.5	5.61	2.01	9.22	7.54	2.91	0.303	0.109	0.818	0.316
A03	1,000	6.03	3.90	288	6.46	6.15	0.006	0.004	0.022	0.021
A04	561	4.19	2.56	150	6.76	4.88	0.007	0.005	0.045	0.033
A05	103	3.05	3.50	34.2	5.37	4.32	0.030	0.034	0.157	0.126
B01	113	5.73	2.96	52.6	11.3	6.63	0.050	0.026	0.215	0.126
B02	107	3.76	2.02	48.6	6.56	4.75	0.035	0.019	0.135	0.098
B03	17.7	2.63	4.28	29.3	4.19	7.17	0.149	0.242	0.143	0.245
B04	47.8	4.11	2.18	45.7	5.67	3.37	0.086	0.046	0.124	0.074
B05	300	6.58	5.54	88.4	8.02	6.62	0.022	0.018	0.091	0.075
Mean							0.086	0.059	0.239	0.145
SD							0.096	0.073	0.266	0.113
Median	105	4.65	2.85	47.2	6.66	4.90	0.043	0.030	0.139	0.112
Min	17.7	2.63	2.01	9.22	4.19	2.91	0.006	0.004	0.022	0.021
Max	1,000	6.58	5.54	288	11.3	7.17	0.303	0.242	0.818	0.332

Table 5: Bioavailability of 1.1 and 2.2 mg administered sublingually compared to 0.2 mg intravenously for individual subjects.

Parameter	State	Mean	SD	%CV	Geometric Mean	Median	Minimum	Maximum
C_{max} (ng/mL)	fasted	6.98	2.51	36.0	6.61	6.28	4.42	12.2
	+ water	7.48	2.91	39.0	7.03	6.48	4.10	11.7
	+ meal	5.28	2.41	45.6	4.80	5.36	2.44	8.16
Ratio for water/fasted		1.072			1.064	1.033		
Ratio for meal/fasted		0.757			0.726	0.854		
T_{max} (hr)	fasted	0.484	0.380	78.7	0.356	0.334	0.167	1.00
	+ water	0.433	0.149	34.4	0.402	0.500	0.167	0.500
	+ meal	0.234	0.149	63.8	0.208	0.167	0.167	0.500
Ratio for water/fasted		0.896			1.129	1.499		
Ratio for meal/fasted		0.483			0.585	0.501		
AUC_{0-t} (ng•hr/mL)	fasted	11.4	3.2	27.8	11.0	10.8	6.41	15.8
	+ water	10.6	2.5	23.3	10.3	10.0	7.36	13.8
	+ meal	8.59	3.60	41.9	8.04	6.47	5.54	13.8
Ratio for water/fasted		0.929			0.944	0.930		
Ratio for meal/fasted		0.755			0.734	0.602		
$AUC_{0-\infty}$	fasted	12.0	3.2	27.0	11.6	11.3	6.65	16.0

Table 6: Pharmacokinetics comparison of fasted with no water, water 10 minutes after administration, and administration 30 minutes after a high fat meal.

safety including physical examination and neurological assessment, lab tests (including hematology and chemistry), ECG, vital signs and AE inquiry. Safety data were collected using AE inquires, measurements of the safety variables and subjects' reports.

The main safety outcomes in this study showed that only one adverse event was recorded, a high CPK value. Investigation revealed that this AE was not related to the study treatment. There were no serious adverse events or deaths in this study. All safety measurements, assessments and analyses revealed that CRLS035 had no effect on physical, neurological or bio-chemical functions. Our results suggest that CRLS035 has a very high safe profile in the limit of the sample size of this study.

Discussion

Flumazenil is currently available only for intravenous (IV) injection and in a low concentration formulation, a fact that limits its use for some important indications [9]. However, it was found to be effective when used by other routes of administration such as oral [10-12], intranasal [13,14], intraoral injection [15], endotracheal [16], and sublingual [17]. The onset of flumazenil action following IV injection is rapid and effective as shown for the treatment of HE [6,7]. In this study the bioavailability of sublingual CRLS035 PK of sublingual CRLS035 in a single dose was 14% and 11% for dosages of 1.1 mg and 2.2 mg, respectively, which correspond to 0.15 mg and 0.24 mg of flumazenil. These are close values to the IV dose of 0.2 mg which is the usual adult dose shown to reverse sedation [9]. This indicates that the total exposure is similar in all three cases. Therefore, the concentrations used in this study are highly bioequivalent to the IV doses. Interestingly enough, flumazenil level was higher in any time-point compare to the intravenous administration for the except the first 10 min (NS).

There are several potential clinical applications for sublingual flumazenil, as we showed earlier in the setting of post-anesthesia [9]. The potential use to immediately reverse symptoms of HE is in great need under specific clinical situations. This may include patients with subclinical HE or patients with a higher grade of encephalopathy who need to perform special tasks. The easier availability by a sublingual route may alleviate its use.

From the safety point of view, this relatively small clinical trial demonstrated that sublingual CRLS035 is safe and harmless. In summary, this study shows that the pharmacokinetics of sublingual flumazenil yields a concentration that is comparable to the intravenous approach and the drug is safe. The sublingual approach would allow convenient and better treatment availability for patients with hepatic encephalopathy as well as for reversing the residual hypnotic effect after surgical procedure.

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