A Randomised, Single-Dose, Two-Period, Cross-Over Phase I Pharmacokinetic Study to Compare TDS®-Diazepam with Rectal Diazepam in Healthy Adult Subjects.

The HypoSpray®Transdermal Delivery System (HTDS) delivers drugs through the skin i.e. lidocaine and testosterone. In this study, the HypoSpray TDS delivers diazepam through the skin, thereby facilitating the safe, rapid and convenient administration of diazepam.

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Abstract

The HypoSpray Transdermal Delivery System (HTDS) is a proprietary liquid formulation that can be applied to intact skin via a metered pump spray to facilitate drug delivery to the circulation.

Objectives

The aim of this study was to assess the ability of the HTDS preparation to deliver diazepam systemically, and to characterize the pharmacokinetic profile of the drug in healthy adult subjects.

Design

We conducted a randomized, single-dose, two-period, crossover phase I (pharmacokinetic) comparative study in twelve healthy volunteers.

Setting

All volunteers received both 10mg HTDS-diazepam topically to the upper chest and 10mg of the rectal diazepam preparation (Diastat®, 10mg diazepam gel), with a minimum washout of 14 days between dosing episodes.

Participants

Twelve healthy males and females (three males, and nine females) out of thirteen recruited subjects completed the protocol in this study.

Main outcome measures

Both formulations were well tolerated in all volunteers. Following topical application of HTDS-diazepam, the mean $AUC_{0\text{-}72h}$ was 1241ng/mL.h and the C_{max} 34ng/mL. The values for rectal Diastat were 4109ng/mL.h and 300ng/mL respectively.

Results and Conclusions

This proof of concept study demonstrates that the HTDS preparation successfully delivered diazepam systemically to adults. As expected, the concentration of diazepam following the HTDS application was lower and not bioequivalent to rectal gel. Future development of this unique system is required.

Introduction

Diazepam (7-chloro-1-methyl-5-phenyl-1,3 dihydro-2H-1,4-benzodiazepin-2-one) is a psychotropic, with sedative and hypnotic effects, muscle relaxant, anticonvulsive activity and in the management of alcohol withdrawal syndrome, anxiolytic, agitation, and amnesic properties. Its actions are mediated by enhancement of γ-amino butyric acid (GABA) activity. Diazepam binds to specific receptors in the central nervous system that have close functional connection with receptors of the GABA-ergic transmitter system resulting in the inhibitory effect of GABA-ergic transmission.

Diazepam may be administered orally, intravenously (IV), intramuscularly (IM), or rectally. Oral diazepam has a rapid absorption and fast onset of action, especially in the fasting state, which confers a rapid and nearly complete absorption (Ochs HR, 1982). In fact, the oral route is the most frequent for therapeutic use, accidental poisonings and abuse. However, it is inappropriate to administer diazepam orally during seizures. While, the IM route may currently be the only practical approach, it has no apparent pharmacokinetic advantage over the oral route. Serum plasma concentration of diazepam in six healthy subjects followed by diazepam 20 mg IV, IM, and orally were 1600, 290, 490 ng/mL respectively, and t_{max} were 15, 60, 30 minutes respectively (Martindale, 1982), and C_{max} reached after 95 minutes in healthy volunteers, and between 1–24 hours in adult patients. In patients with active seizures, the IV route may be used for cessation of seizures - having a rapid onset of action (1–5 minutes); yet this method is by no means without risk, hence facilities for protecting the airway and reversing respiratory depression with mechanical ventilation should be at hand. Venous thrombophlebitis can be reduced by using an emulsion (Diazemuls®), but at the expense of a lower plasma concentration than for IV (Valium®) (Fee et al., 1984). This could not be confirmed by Naylor and Burlingham

(Naylor and Burlingham, 1985). Similarly, the rectal route can be used to treat acute repetitive seizures. Rectal diazepam can be administered relatively easily and safely by non-medical personnel; however, suppositories generally have less predictable bioavailability as a result of slow and erratic absorption. Administration of rectal solutions and gels have their own problems which include leakage of active ingredient through the cracks as recently found in the tips of the 10 and 20 mg Diastat® AcuDial® gel prefilled syringes, the consequence of which was an FDA alert. Leakage of gel/solution may result in reduced delivery of active diazepam, and thus less than that required for effective treatment of acute repetitive seizures, ultimately increasing the incidence of more serious problems, including status epilepticus. The potential of transdermal diazepam has been previously studied in vitro and demonstrated as not effective for the penetrate of human skin (Koch et al., 1987).

We have developed a transdermal drug delivery system (HTDS, Transdermal Delivery Solutions Corp, Florida, USA), which is a liquid formulation that can be combined with the drug entity to form a novel and more convenient pharmaceutical dosage form (spray form), to enhance drug delivery through the skin. The spray provides a fast, simple, easy, and effective dose delivery without leaving any residue. Recent studies of the HTDS for lidocaine and testosterone administration have reported no side effects, and resulted in, a), acceptable anaesthesia five minutes post-application of TDS-Lidocaine (Tucker et al., 2006), and b), bioequivalence of HTDS-Testosterone to AndroGel® (Chik et al., 2006).

Results

Twelve healthy subjects successfully completed the protocol. Nine subjects were female, and three subjects were male. The mean (SD) age of the subjects was 28.3 (8.4) years, and the mean (SD) BMI was 24.2 (5.0) kg/m^2.

Figure 1 and Figure 2 show the plots of mean plasma concentration of diazepam and desmethyl-diazepam versus time. Concentrations of diazepam were higher for the rectal formulation (Diastat®) in all of the subjects compared to HTDS-Diazepam (spray formulation). The pharmacokinetic parameters AUC, C_{max}, and t_{max} are listed in Table 1 for both treatments. The AUC and C_{max} values were calculated for 0–72h. The mean $AUC_{0\text{-}72h}$ and C_{max} were lower following application of HTDS-diazepam (1241.3 ng/mLh) and (34.3 ng/mLh), compared with Diastat® (4108.5 ng/mLh) and (300 ng/mLh) respectively (Table 2). The 90% confidence intervals on the relative difference of the ratio for the $AUC_{0\text{-}72h}$ and the C_{max} between HTDS-diazepam and Diastat® were not within the bioequivalence limit (80, 125%). Based on the 90% CI of diazepam ratio C_{max} 7.3–14%, $AUC_{0\text{-}72h}$ 19.7–37.6% desmethyl-diazepam ratio (C_{max} 37.6–53.8%, $AUC_{0–72h}$ 33.4 –57.8% were not within the bioequivalence limit (80–125%).

Discussion and Conclusion

No serious or unexpected adverse events were reported or observed during the study. The drug formulations and protocol requirements were well tolerated by all subjects.

The HTDS-Diazepam preparation was shown to be able to deliver diazepam systemically in human subjects. However, the maximum concentration of diazepam and desmethyl-diazepam following HTDS-Diazepam administration were found to be ≈ 11% equivalent to rectal diazepam (Diastat®). The results from this study showed that while diazepam was absorbed transdermally, HTDS-Diazepam was not bioequivalent to rectal diazepam (Diastat®).

The plasma concentration of diazepam needed to stop convulsions is not well established, although a range of 150–350 ng/mL has been suggested (Agurell et al., 1975, Knudsen, 1977). A study by Ogutu et al following rectal administration of diazepam not only failed to terminate all

of the convulsions but also reported variable plasma drug concentrations over time (112–1953 ng/mL, 0.17–36 h) compared with the IV group (402–1507 ng/mL, 0.42–3.13 h) (Ogutu et al., 2002). Additionally, the recurrence of seizures in two children was observed, at diazepam plasma levels of 150–200 ng/mL (Agurell et al., 1975). The pharmacokinetic characteristics have also been studied for oral diazepam, which were highly variable even in a relatively homogeneous population (Greenblatt et al., 1989).

Similarly, in this study, Diastat® only achieved the suggested effective plasma concentration (138.3–378.7 ng/mL) of the range 150–350 ng/mL in ten of 12 subjects; probably due to diazepam metabolism variability between individuals, which could explain the results of the Ogutu et al study. Desmethyldiazepam was not quantified in all children who received diazepam IV but it was measured in three children who received rectal diazepam. Diazepam kinetic response to a particular dose seems to vary broadly between subjects, an observation noted in Ogutu: "One child had a plasma diazepam concentration below 200 ng/mL five minutes following IV administration. Even after a repeat dose of diazepam 30 minutes later, plasma diazepam concentrations in this child declined rapidly from 414 ng/mL at five minutes after the repeat dose to less than 200 ng/mL within 20 minutes. Two children who received diazepam PR also failed to achieve a plasma concentration of 200 ng/mL. Despite not achieving this target, all three children stopped convulsing. Although convulsions recurred within 30 minutes in each child."

Due to the presence of active metabolites, the serum/plasma concentration value of diazepam alone is not useful in predicting the effects of the drug. Diazepam and metabolites were analysed, and only diazepam and desmethyl-diazepam were assayed in all subjects for both treatments, (i.e. diazepam was metabolized in both dosage forms). Desmethyl-diazepam is an active sedative,

which is excreted by the kidneys. A study by Nicholson and co-workers has reported that desmethyl-diazepam (10 mg) can improve sleep compared to clorazepate (15 mg) (Nicholson et al., 1976). Furthermore, in one subject in the HTDS treatment arm, no diazepam was observed, however desmethyl-diazepam was present. This may be due to the rapidity of skin metabolism of diazepam, or accumulation of diazepam in subdermal adipose tissue.

Future development of this novel system will focus on staged adjustments to the HTDS formulation, dose and/or concentration of the HTDS-diazepam system, in order to attain the therapeutic concentration range with the aim of developing a more convenient alternative to rectal or intravenous diazepam treatments. Additionally, we may need to further consider the influence of diazepam metabolism in the skin (there are numerous enzymes beneath the Stratum Corneum in the Viable Skin (Guy et al., 1987). The entire skin-to-liver metabolism ratio has been suggested to be 0.8-2.4 for different enzyme systems (Noonan and Wester, 1985) slowly penetrating compounds, ones possessing lipophilic characteristics like diazepam might be expected to be metabolized more completely.

The presence of desmethyl-diazepam concentrations in the first time point after the administration of the HTDS system in five subjects suggested that desmethyl-diazepam had a half-life of more than 14 days in this study.

This suggests that in future studies concerned with the bioequivalence, absorption, and bioavailability of topically applied drugs, skin metabolism of the drug needs to be considered.

Methods

Study materials

The HTDS was supplied by TransDermal Technologies, Inc., Florida, USA predecessor to Transdermal Delivery Solutions Corp, and diazepam was added to form TDS-diazepam (batch number CT2007007) prepared and supplied by the Royal London Hospital, Pharmacy Department. HTDS-diazepam was supplied as a liquid formulation, delivered by metered pump spray, with each spray containing 2mg diazepam. Diastat® was supplied as a gel in unit-dose, containing 10mg diazepam.

Study design and treatments

We conducted a randomized, single-dose, two-period, crossover phase I (pharmacokinetic) comparative study in twelve healthy male and female volunteers. All volunteers received both 10mg HTDS-diazepam topically to the upper chest and 10 mg of the rectal diazepam preparation (Diastat®, 10mg diazepam gel), with a minimum washout of 14 days in between dosing.

Subjects

Twelve healthy subjects were required to complete the protocol. The study was approved by St Thomas Hospital Research Ethics Committee, and by the MHRA (Medicines and Healthcare Products Regulatory Agency), UK. The subjects were to give written informed consent before taking part in the study, and each was to fulfill all entry criteria based on physical examination, medical history, and clinical laboratory tests.

Study protocol

On the morning of each study day, blood pressure, heart rate, respiratory rate, and body temperature were measured after subjects had rested for 10 minutes. A 20G cannula was placed in a large antecubital vein for blood collection. The drug formulation was then applied to the

chest and gently rubbed into the skin for the HTDS-diazepam; the Diastat® was administered rectally. Regular meals and beverages were provided throughout the study day, breakfast at 07:30 am, lunch at 1pm, and dinner at 8 pm. After dosing, subjects were permitted to engage in normal daily activities. Approximately 4 ml of blood was collected at 0 h (immediately prior to dosing), 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24, 32, 48, 72 h post dose. Samples were added to EDTA-containing tubes to clot at room temperature (26°C) and were then centrifuged at 3000g for 10 minutes. The plasma was transferred to labeled tubes and stored at -20°C until analysis.

Diazepam analysis

Diazepam and metabolite concentrations were measured in plasma samples using a high-performance liquid chromatographic assay, with mass-spectrometric detection, (HPLC/MS). The assays were performed at the Analytical Unit, St George's, University of London, using a liquid/liquid extraction method. The methods were calibrated to demonstrate adequate prediction, specificity, recovery, accuracy and precision (within and between assays).

The limit of quantification was < 5.0ng/mL for the assay. All the study samples were analyzed together with quality control samples containing three different concentrations. The coefficients of variation for imprecision for diazepam concentration at 20 ng/mL was 2.53%, at 200 ng/mL was 2.76%, and at 750 ng/mL 0.4%, and largest absolute value for inaccuracy was 2.8%.

Data analysis

Pharmacokinetic parameters were determined and the statistical analysis was performed using Windows Excel 2007. C_{max} and t_{max} were determined directly from the individual plasma concentration-time curves, and the AUC was calculated using the linear trapezoidal method. The

difference between treatments for $AUC_{0\text{-}72h}$ and C_{max} were analyzed after logarithmic transformation using analysis of variance (ANOVA) for crossover studies, which accounts for variation due to sequence, subject, formulation, and period. Bioequivalence testing was based upon the 90% confidence interval (CI) for the ratio of population means between the two treatments. This method is equivalent to the corresponding two one-sided test procedures, with the null hypothesis of bioequivalence at the 5% significance level. For formulations to be bioequivalent, the ratio (test: reference) must fall between the 0.8–1.25 confidence interval (Nation and Sansom, 1994, FDA, 2001, EMEA, 2001).

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Tables and figures

Table 1 Bioequivalence parameters for diazepam and desmethyl-diazepam, HTDS diazepam (Test formulation, A) versus rectal diazepam (Reference formulation, B)

		-90% CI	Point Estimate%	+90% CI
Diazepam	t_{max}	1.63	2.13	6.75
	C_{max} (A:B)	7.3	10.1	14.0
	C_{max} (B:A)	715.8	990.1	1367.7
	$AUC_{0\text{-}72h}$ (A:B)	19.7	27.2	37.6
	$AUC_{0\text{-}72h}$ (B:A)	266.1	367.6	507.8
Desmethyl-diazepam	t_{max}	-20	-4	12
	C_{max} (A:B)	37.6	45.0	53.8
	C_{max} (B:A)	186.0	222.4	266.0
	$AUC_{0\text{-}72h}$ (A:B)	33.4	44.0	57.8
	$AUC_{0\text{-}72h}$ (B:A)	172.9	227.5	299.4

Table 2 Derived diazepam and desmethyl-diazepam Geometric mean (CV percentage) for rectal and HTDS diazepam (10 mg)

	Rectal t½	Rectal t_{max}	HTDS t_{max}	HTDS - Rectal Difference	Rectal C_{max}	TDS C_{max}	TDS/Rectal Ratio
Diazepam	59.2	1.0	24.0	10.1%	3929.3	1104.5	27.2%
	(121.7)	(24.3)	(56.0)	(49.6)	(25.9)	(42.9)	(40.8)
Desmethyl -diazepam	-	33.0	14.8	45.0%	1714.9	753.8	44%
		(29.8)	(36.2)	(33.7)	(37.2)	(46.1)	(43.6)

Figure 1 Mean plasma diazepam versus time in 12 subjects following a 10 mg dose rectally (filled red circles) and transdermally by HTDS diazepam (filled blue squares), logarithmic concentration axis.

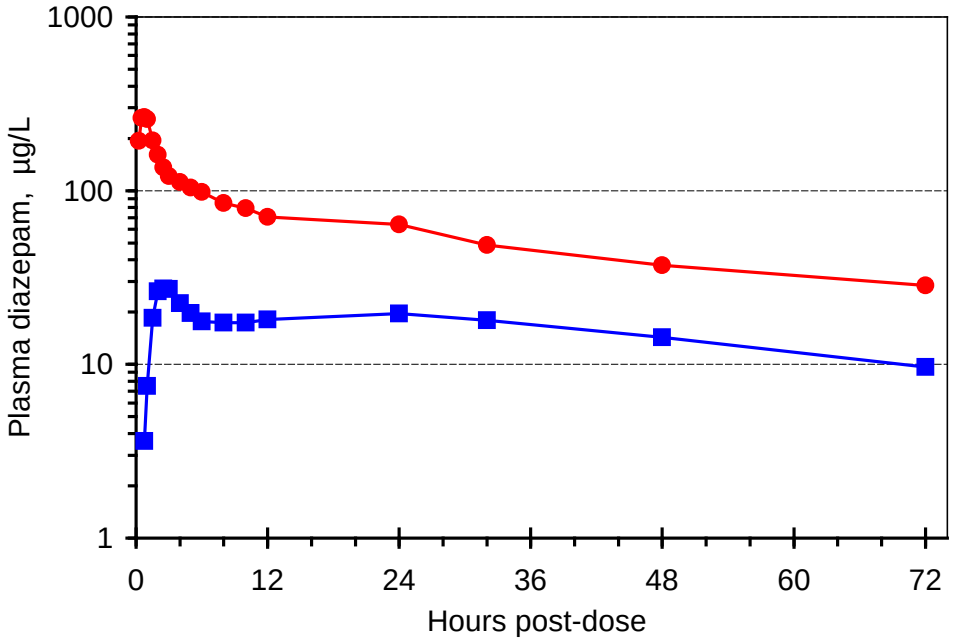
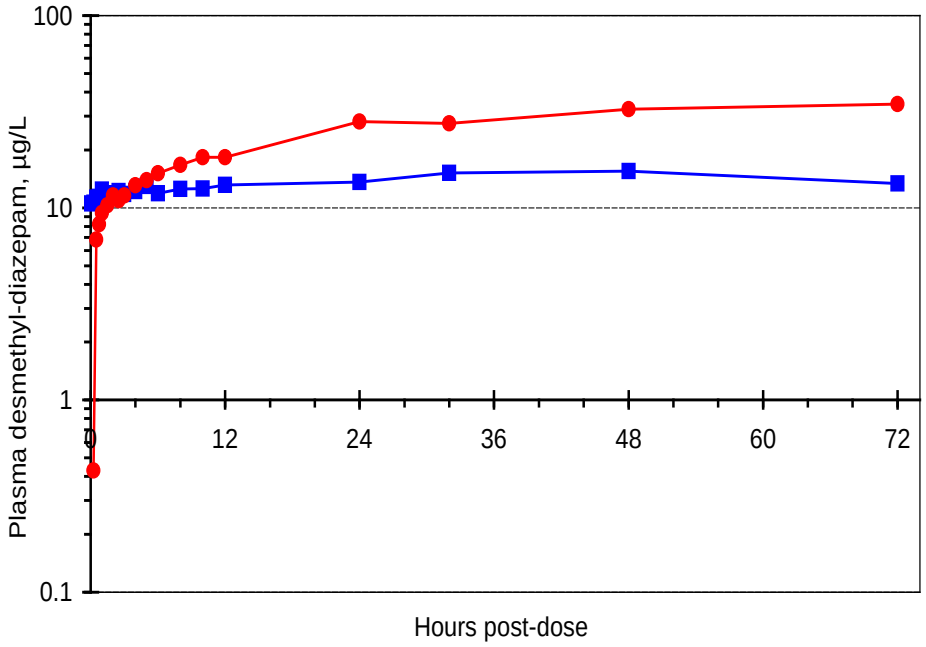


Figure 2 Mean plasma desmethyl-diazepam versus time in 12 subjects following a 10 mg dose rectally (filled red circles) and transdermally by HTDS diazepam (filled blue squares), logarithmic concentration axis.



List of Abbreviation	Full name
am	Before midday
ANOVA	Analysis of variance
AUC	Area under the curve
BBB	Blood brain barrier
BMI	Body mass index
BP	Blood pressure
CI	Confidence interval
C_{max}	Maximum plasma concentration
CV	Coefficient of variation
EDTA	Ethylene diamine tetraacetic acid
EMEA	European medicines evaluation agency
FDA	Food and drug administration
g	Gram
GABA	γ-amino butyric acid
HPLC	High performance liquid chromatography
h	Hour
i.g	For example
IM	Intra muscular
IV	Intra venous
kg	Kilogram
m^2	Square meter
mg	Milligram
mL	Millilitre
MS	Mass spectrometric
MHRA	Medicines and healthcare products regulatory agency
ng	nanogram
pm	After noon
QMUL	Queen Mary University of London
SD	Standard deviation
$t_{1/2}$	Plasma concentration half life
HTDS®	Proprietary HypoSpray® transdermal drug delivery system
t_{max}	Time passed since administration at which the maximum plasma
US&USA	United States of America