Research Article

Pharmacokinetic Evaluation of Intranasally Administered Vinyl Polymer-Coated Lorazepam Microparticles in Rabbits

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Abstract. The intranasal (IN) administration of lorazepam is desirable in order to maximize speed of onset and minimise carry-over sedation; however, this benzodiazepine is prone to chemical hydrolysis and poor airway retention, and thus, innovative epithelial presentation is required. The aim of this study was to understand how the *in situ* self-assembly of a muco-retentive delivery system, formed by the dissolution of vinyl polymer-coated microparticles in the nasal mucosa, would influence lorazepam pharmacokinetics (PK). IN administration of the uncoated lorazepam powder (particle size, $6.7 \pm 0.1 \mu\text{m}$) generated a biphasic PK profile, which was indicative of sequential intranasal and oral absorption ($n=6$; dose, 5 mg/kg). Coating the drug with the vinyl polymer, MP1 ($9.9 \pm 0.5 \mu\text{m}$ with $38.8 \pm 14.0\%$, w/w lorazepam) and MP2 ($10.7 \pm 0.1 \mu\text{m}$ with $47.0 \pm 1.0\%$, w/w lorazepam), allowed rapid systemic absorption (MP1, T_{max} 14.2 ± 4.9 min; MP2, T_{max} 9.3 ± 3.8 min) in rabbits and modified the PK profiles in a manner that suggested successful nasal retention. The poly(vinyl pyrrolidone)-rich MP2 system provided the best comparative bioavailability, it prolonged the early-phase nasal drug absorption and minimised drug mucociliary clearance, which correlated well with the intermolecular hydrogen-bond-driven vinyl polymer interactions observed *in vitro*.

KEY WORDS: intranasal; lorazepam; microparticles; pharmacokinetics; poly(vinyl alcohol).

INTRODUCTION

Lorazepam is a benzodiazepine that is used as a hypnotic, sedative and anxiolytic. In acute clinical situations, it is commonly administered via the intramuscular or intravenous route because oral dosing provides an inadequate speed of onset, but there is a growing demand to find a non-invasive, rapid action formulation for this molecule. Several intranasal (IN) drug delivery strategies have been developed for lorazepam because nasal epithelial presentation allows easy access, rapid absorption, reduced first-pass metabolism and the potential to minimise carry-over sedation by facilitating rapid exposure to the systemic circulation (1–7). However, the physicochemical properties of lorazepam, like

many benzodiazepines, are not well suited to IN delivery (8). Lorazepam is ionised in physiological fluids ($\text{pK}_a=1.3$, 11.5 at 20°C), but it retains its lipophilicity ($\log P_{\text{octanol/pH } 7.4}=2.4$), and hence, it is transported passively across the epithelium at an acceptable rate (9). Its delivery is mainly limited by its low water solubility (0.08 mg/ml) (10), low potency and susceptibility to hydrolysis in aqueous solution (11).

Previous *in vivo* pharmacokinetic (PK) evaluations of IN lorazepam have presented the molecule to the nasal epithelium as either a glycol solution or colloid suspension (1,4,12,13). Instilling a lorazepam solution into the nose has been shown to result in rapid clearance and a pharmacokinetic profile that demonstrates the traits of significant secondary oral absorption, and hence, this presentation does not satisfy the arising clinical need (14). Encapsulating lorazepam in a colloid, such as a phospholipid-containing vesicle or including it within a cyclodextrin, can modify the drug's PK profile (1,11). These changes in absorption may be a consequence of direct transport of the colloid across the nasal mucosa (15–17). If particles do penetrate the epithelium, the surface properties of the particle dictate bioretention, or particle surface-induced mucoadhesion, but toxicity issues associated with particle uptake have yet to be resolved, and hence, colloidal nasal formulations have not yet gained widespread commercial acceptance (18,19). The maintenance of physical and chemical stability and the use of preservatives are just some of the issues that hinder the successful

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production of IN colloidal sprays. The formulation of a microparticle carrier, which can be directly aerosolised into the nose, seems an attractive alternative to systems that use a liquid vehicle in the case of lorazepam; however, producing a system that allows adequate drug loading, rapid dissolution and bio-retentive matrix self-assembly in the nasal mucosa is not trivial.

Buttini *et al.* recently employed biocompatible vinyl polymers to engineer drug-rich beclomethasone 17-valerate and budesonide microparticles which appear to have the characteristics to meet the demands of bio-retentive IN delivery (20). In subsequent work, Traynor *et al.* used the same particle engineering method to generate a lorazepam microparticle (21). Layering the lorazepam surface with vinyl polymers influenced the dissolution rate and aerosolisation properties by controlling the drug's cohesion and interactions with water. However, using the *in vitro* methods outlined in this previous work it remained unclear if such a formulation approach could facilitate bio-retention and minimise secondary oral absorption effects which have previously been shown to be an issue with this molecule when administered IN (14). The aim of this study was to evaluate the influence of presenting lorazepam as a vinyl polymer-coated microparticle upon the relative bioavailability and pharmacokinetics *in vivo*. In order to test the hypothesis generated *in vitro*, that the intermolecular hydrogen bonding of the vinyl polymers dictated the post-deposition behaviour of encapsulated lorazepam, two types of polymer-coated microparticles were employed in the study. The level of poly(vinyl pyrrolidone) (PVP) was varied in the vinyl polymer coating of the two particles as this is known to plastisize the vinyl alcohol (PVA), releasing the OH moiety from the highly ordered intramolecular bonding, which effectively activates the polymer's ability to form new interactions with the nasal mucosa (22).

MATERIALS

Ammonium solution (25%) was supplied from BDH (Poole, UK). Potassium dihydrogen orthophosphate and high-performance liquid chromatography (HPLC)-grade water, methanol, ethanol, acetonitrile, orthophosphoric acid and cyclohexane were from Fisher (Leicestershire, UK). Poly(vinyl alcohol) (PVA 23-88) and PVP (Kollidon® 17) were supplied by KSE (Troisdorf, Germany) and BASF (Wantage, UK), respectively. Formic acid, 1-chlorobutane, sodium chloride, sodium dodecyl sulphate (SDS), lorazepam and temazepam were purchased from Sigma-Aldrich Ltd. (Gillingham, UK).

METHODS

Formulation Preparation

To facilitate oral administration, the lorazepam was prepared as an aqueous suspension (LS) by weighing approximately 200 mg lorazepam and suspending it in 10 ml HPLC-grade water with the help of gentle magnetic agitation for 24 h while protecting it from light at ambient temperature ($23 \pm 2^\circ\text{C}$). Lorazepam is prone to hydrolysis, but it has been previously shown to be stable for 24 h in water, and no degradation was evident in the HPLC chromatograms (23). IN lorazepam administration employed the powdered

material as received from the supplier (LP). Two types of lorazepam-loaded microparticles (MP1 and MP2) were prepared using a previously published method (21). Approximately 1.5 g lorazepam was weighed and suspended in an aqueous solution (150 ml) containing 0.15 g PVP and PVA (0.9 g for MP1 and 0.09 g for MP2) to produce suspensions. These suspensions were spray-dried using a 191 bench top spray dryer from Buchi Labortechnik AG (Postfach, Switzerland). During spray drying, the suspensions were held under constant agitation by magnetic stirrer. The inlet temperature was 180°C , with an air flow of 650 ml/h, an atomisation flow of 70% and a feed rate of 3 ml/min. The dried particles (MP1 and MP2) were collected and stored under phosphorus pentoxide until required (<2 months).

Formulation Characterization

The lorazepam content of the spray-dried formulations (MP1 and MP2) was tested by HPLC. Briefly, a 10-mg aliquot of each formulation was dissolved in a 100-ml ethanol and water mixture (1:1, *v/v*), and then the solution was further diluted in an appropriate ratio using the same solvent prior to HPLC analysis. Drug content in each formulation was calculated by dividing the drug amount by the formulation mass, and data were presented as mean $\pm$ standard deviation ($n=6$). The lorazepam-loaded microparticles (MP1 and MP2) and pure lorazepam particles (LP) were characterized in terms of hydrodynamic diameter using a laser diffraction system (Mastersizer X, Malvern, UK). The lens used was 100 μm with active beam length at 14.3 mm, and the sample unit was a magnetically stirred cell (MS-7). Approximately 2 mg of MP1 or MP2 was dispersed in 1 ml lorazepam-saturated cyclohexane and sonicated in a water bath (Model F5100b, Decon Laboratories, UK) for 10 s to disperse any possible agglomerates. The samples were then added dropwise into the MS-7 cell containing drug-saturated cyclohexane with continuous magnetic stirring until an ideal obscuration (10–30%). Measurement was repeated eight times for each sample and three samples were measured per batch using a randomized sampling procedure. By modifying a method that was described in the United States Pharmacopoeia (USP 30), lorazepam release from drug-loaded microparticles (MP1 and MP2) was monitored in SDS aqueous solution (0.2%, *w/v*) at 37°C . The dissolution vessels were filled with a 500-ml SDS aqueous solution, and the rotation speed of the apparatus was 100 rpm. A known weight of lorazepam alone or spray-dried formulations were applied as a thin layer to a piece of double-sided sellotape which was attached to a small metal disc. The metal disc was screwed directly on to the end of the stirring rods and inserted into the dissolution vessel. At predetermined time intervals, 1 ml of sample was withdrawn for HPLC assay, and the same volume of pre-warmed dissolution media was replaced immediately.

Lorazepam HPLC Assay

Quantitative analysis of lorazepam was performed using a reverse-phase HPLC system consisting of an autosampler (Waters 717 plus), a pumping controller (Waters 600E), a dual lambda absorbance detector (Waters 2487) coupled with Millennium32 Software, version 4.0 (Waters, Milford, MA,

USA). A Chromolith® RP-18e (100×4.6 mm) column with a Chromolith® RP-18e guard cartridge (5×4.6 mm) was used for separation. The mobile phase consisted of 75% (v/v) water (pH 2.1, adjusted with orthophosphoric acid) and 25% (v/v) acetonitrile containing 35 mM potassium dihydrogen orthophosphate with a flow rate of 2 ml/min. The needle wash was performed using a mixture of methanol and water (90:10, v/v). The injection volume was 10 µl, and detection was at 220 nm resulting in a retention time of 12 min for lorazepam. The assay range was 0.2 to 20 µg/ml ($r^2 > 0.99$); the limit of detection (LOD) and limit of quantification (LOQ) were 0.26 and 0.88 µg/ml, respectively.

Pharmacokinetic Study

With approval from the Northwick Park Hospital Clinical Ethics Committee, UK (PPL 80/1804 24) New Zealand white rabbits (3.3±0.2 kg, Harlan, UK) were randomly divided into four groups. The animals were housed in individual cages with free access to food (Lillico, UK) and fresh tap water at a constant temperature of 20°C with a 12:12 h light–dark cycle. Four formulations (LS, LP, MP1 and MP2) were assessed as detailed in Table I (six animals per formulation). All animals were dosed early morning and culled by a schedule 1 method upon termination of the experiment. A single dose of pure lorazepam powder (LP) and the drug-loaded microparticles (MP1 and MP2) was administered IN using an adapted Penn-Century Dry Powder Insufflator™ (Penn-Century, Inc., Philadelphia, PA, USA), whilst lorazepam aqueous suspension (LS) was administered via oral gavage. At predose ($t=0$), and at 5, 10, 15, 20, 30, 60, 90, 120, 240, 360 and 480 min after administration, a 1-ml blood sample was collected from the carotid cannula into heparinised microcentrifuge tubes and immediately immersed in an ice bath. Plasma samples were prepared by centrifugation of the blood (5,800×g for 10 min) at 4°C, aspiration into cryovials and snap freezing in liquid nitrogen. The samples were stored at –80°C prior to analysis. A liquid–liquid extraction was performed using a 200-µl aliquot of plasma and temazepam as an internal standard (100 ng/ml). A 20-µl aliquot of 25% ammonia solution mixed with 1,090 µl *n*-butyl chloride, organic phase, was added to the tubes, mixed for 5 min and centrifuged for 10 min at 5,000 rpm using an Eppendorf MiniSpin centrifuge (Harmburg, Germany). The samples were stored upright at –20°C until the water phase was frozen, and this was separated from the unfrozen organic layer. The organic solvent was removed by evaporation, and a methanol and water mixture (60:40, v/v) was used to reconstitute the samples which were analysed by high-performance liquid chromatography–mass spectrometry. An

Agilent HP1100 HPLC system (Agilent Technologies, Stockport, UK) interfaced directly to Micromass Quattro LC triple quadrupole mass spectrometer (Waters, Manchester, UK) with electrospray ionisation in positive mode was employed as the analytical system. The capillary voltage was set to 2.8 kV. The source block and desolvation temperatures were maintained at 100°C and 450°C, respectively, and data processing (using precursor and product ions: 321/275 (lorazepam); 301/255 (temazepam)) was performed by the MassLynx v. 3.5 software (Waters Corp., Milford, MA, USA). HPLC separations were performed on XTerra MS C18 3.5-µm column (150×2.1 mm) from Waters Corp. (Milford, MA, USA); isocratic elution was carried out with the mobile phase containing 0.1% (v/v) formic acid in methanol/water (60:40, v/v) at a flow rate of 0.2 ml/min. The injection volume was 10 µl and the run time was 10 min. Retention times were approximately 5.7 and 6.7 min for lorazepam and the internal standard temazepam. The assay LOD was 0.28 ng/ml and the LOQ was 0.98 ng/ml using a range of standards shown to maintain linearity between 1 and 1,000 ng/ml ($r^2 > 0.99$).

Data Analysis

The concentrations of lorazepam in plasma samples were calculated based on the calibration curve over the range of 1–1,000 ng/ml. Peak ratios between lorazepam and temazepam were employed to normalise for the extraction procedure. Pharmacokinetic data was fitted using WinNonLin (v.5.0.1, Pharsight, Phoenix®, USA). Values for $C_{\max}$ (maximum drug concentration in the plasma), $T_{\max}$ (the period of time required to achieve $C_{\max}$), AUC_{∞} (area under the plasma concentration time curve to the infinite) and AUC_{0-20} (area under the plasma concentration time curve to 20 min) were determined for the average obtained data. The relative bioavailability of the formulations was calculated by dividing AUC_{∞} of one formulation by the AUC_{∞} of the second as the doses were equivalent. All data were statistically analysed using SPSS v.16 (SPSS Inc., Chicago, IL, USA). One-way ANOVA or Student's *t* test was used, and the confidence interval was chosen to be 95%. When appropriate, a post hoc test (Tukey's honestly significant difference test) was employed.

RESULTS

Formulation Characterization

The lorazepam content in MP1 (38.8±14.0%, w/w) was significantly ($p < 0.05$) lower than its counterpart MP2 (47.0±

Table I. Summary of the Lorazepam (LZ) Formulations and Dosing Information in the Rabbit Pharmacokinetic Study

Formulation	Composition	Route	Dose (mg/kg)
MP1	6:1 PVA/PVP+LZ	Nasal	5
MP2	0.6:1 PVA/PVP+LZ	Nasal	5
LP (control 1)	LZ	Nasal	5
LS (control 2)	LZ	Oral gavage	5

MP1 and MP2 the two types of spray-dried drug microparticles with vinyl polymer coatings composing of poly(vinyl alcohol) (PVA) and poly(vinyl pyrrolidone) (PVP), LS lorazepam aqueous suspension, LP pure lorazepam particles

1.0%, w/w), but both types of spray-dried polymer-coated particles (MP1 and MP2) displayed a significantly ($p < 0.05$) larger median diameter than the control lorazepam powder (LP); the particle size of LP, MP1 and MP2 was 6.7 ± 0.1 , 9.9 ± 0.5 and 10.7 ± 0.1 μm , respectively. The dissolution of lorazepam from vinyl polymer-coated particles (MP1 and MP2) was significantly ($p < 0.05$) faster than that from pure lorazepam powders. Within 5 min, approximately 90% of drug was released into the dissolution medium from MP1 and MP2, whilst only ca. 50% of drug was available when presented using the LP. Note that these data were previously published (21) and re-presented in this article for ease of reference.

Lorazepam Pharmacokinetics

The delivery of lorazepam using the IN formulations resulted in the rapid appearance of the drug in the systemic circulation of the rabbits, whereas oral administration led to a delay to achieving detectable drug levels (Figs. 1 and 2). Both MP2 and the IN control lorazepam formulation showed a biphasic PK profile, but this was not apparent with the other formulations. Attempts to fit the data to the noncompartmental model (NCA) and the monocompartmental first order, no lag time, first-order elimination (MC) PK models were successful (Table II), but analysis using bicompartmental or multicompartmental models did not result in plausible model fits. Upon consideration of the model correlations, the NCA model was used to derive the PK parameters for formulation comparison (Table III).

IN administration of the uncoated lorazepam particles generated the highest maximum drug concentration (C_{max} , Table III) and the best bioavailability (calculated using AUC_{inf} , Tables III and IV). However, within the first 20 min of the PK study (the nasal mucociliary clearance time has been reported to be approximately 20 min (24)),

Fig. 1. Plasma concentration vs. time profile of the lorazepam aqueous suspension (LS, filled square) administered orally; and pure lorazepam particles (LP, open circle) used as received and administered intranasally. Points represent the mean $\pm$ standard error ($n=6$)

Fig. 2. Plasma concentration vs. time profile of two microparticulate lorazepam formulations administered intranasally: MP1 (filled square) and MP2 (open circle) (MP1 and MP2 represent the two types of spray-dried drug microparticles with vinyl polymer coatings). Points represent the mean $\pm$ standard error ($n=6$)

the AUC (AUC_{20} , Table III) for MP2 was 58% higher than the LP. Comparison of the two vinyl-coated MP formulations showed that MP2 generated a higher C_{max} , lower T_{max} and a superior AUC across the PK profile, which resulted in an enhanced relative bioavailability. Although the calculated T_{max} values suggest that the IN administration of uncoated lorazepam microparticles resulted in a slower drug uptake into the systemic circulation compared to the oral suspension, the biphasic nature of the IN lorazepam suggested sequential nasal and oral epithelium absorption was occurring when the drug was applied to the epithelium without the polymer. The time course of IN absorption after direct nasal administration could be identified as between 10 and 30 min from the MP1 profile, whilst direct oral absorption was assumed to be reflected by the T_{max} of LS. MP2 produced a biphasic PK profile, but the second spike was not consistent with the secondary absorption traits of the comparative lorazepam PK data.

DISCUSSION

In this study, two types of particles were administered to the nasal epithelium of rabbits, pure lorazepam microparticles and vinyl polymer-coated lorazepam microparticles. The pure lorazepam particles were approximately half the size of the polymer-coated particles, and they have been shown to release the drug at about half the speed (21). The higher variability of lorazepam content in MP1 was attributed to the different adsorption behaviour of the vinyl polymers in the solution state. PVP is known to modulate the solvation effects of PVA during drying. Increasing the relative proportion of PVP in the microparticle has previously been shown to generate a less cohesive product, and it is probable that this led to more consistency in the particle homogeneity testing results

Table II. Pharmacokinetic Model Fitting

Formulation	NCA model (R^2)	MC model (R^2)
LS	0.989	0.920
LP	0.758	0.557
MP1	0.966	0.922
MP2	0.835	0.774

The correlations (R^2) to the noncompartmental model (NCA) and monocompartmental first order, no lag time, first-order elimination model (MC) are displayed

MP1 and *MP2* the two types of spray-dried drug microparticles with vinyl polymer coatings, *LS* lorazepam aqueous suspension, *LP* pure lorazepam particles

(20). Differences in aerosolisation were not considered significant in this work as the powder applicator atomised the powders directly onto the epithelium and no significant loss of drug appeared to occur as a consequence of retention in the application device. Using PVA and PVP as the vinyl polymers to coat the particles generates microparticles with high content of active agent and allows a core-shell structure to be generated. As the drug composes the core and vinyl polymers (PVA and PVP) form the outer shell, the polymers dominate the initial processes that occur when the particles hit the mucosal membrane (20,21).

Given the incapability of particles with a size of ca. $>1\ \mu\text{m}$ to cross the nasal mucosa (tight junction pores at this epithelium are $<15\ \text{nm}$, (25)) and the fact that the vast majority of particles in the applied formulations were above this size, drug absorption was assumed to be subject to limitations imposed by particle dissolution, local diffusion and passive transepithelial transport. Although the rate of lorazepam dissolution in the presence of nasal fluids is not known, the dissolution data generated in the presence of SDS that showed more rapid drug release from the larger vinyl-coated lorazepam microparticles compared to the small uncoated material indicated that the particle size of the formulations was not the factor that dominated drug absorption. Furthermore, the enhancement of dissolution in the presence of the vinyl polymers could not simply explain the PK profiles. The dissolution model, which has become known as the Noyes-Whitney equation, outlines a clear relationship between particle size of an administered material

and its dissolution rate (26). This relationship is confounded with the vinyl polymer-coated particles by the presence of the polymer at the solid-liquid interface. However, the hydrophilic vinyl polymer system is known not to act like a classical diffusion-controlled release system described by Higuchi (27); rather, the rich network of alcohol moieties on the surface of the macromolecule encourages surface wetting, the polymer disperses, forms its secondary structure in the aqueous environment and facilitates the interaction of the drug with the polar vehicle. As the release rate of the microparticles is rapid, the polymer matrix does not significantly retard drug diffusion away from the original solid material, and this means that the polymer matrix must be relatively loose; this is ideal to form a mucoadhesive system. The rapid drug release even in the presence of significant levels of the macromolecules (ca. 40–50% of the particle is considered to be the polymer) is likely to generate nasal mucoadhesion, and the PK study results support this hypothesis (28–30).

Lorazepam has no known bioactive metabolites, and it is removed from the body relatively slowly upon phase 2 glucuronide conjugation (31). As a consequence, the PK profile of this agent can be used in conjunction with the *in vitro* data to understand bioretention and epithelial absorption. Bioretention on mucosal surfaces is known to be controlled through the manipulation of the free hydrogen-bond-presenting moieties on the surface of polymer matrices (32). Lorazepam interacts with the vinyl polymers (21), and upon dispersion within the nasal mucosa, the hydrogen bond-forming capacity is controlled by the intermolecular interactions between the drug and the two polymers in the vinyl polymer coating (21). Unlike the uncoated microparticles, which reside on the surface of the epithelia post-deposition and are cleared rapidly by mucociliary clearance, when the drug is administered using the vinyl polymer-coated microparticles, its appearance in the blood will be influenced by how strongly the polymers can anchor the drug to the epithelium in the presence of ciliary clearance of the nasal fluid (32).

The calculated T_{max} values for the MP1 and MP2 particle appeared to demonstrate that the vinyl polymer accounted for the drug being absorbed almost ten times faster compared to the uncoated IN material. However, a close inspection of the PK profiles suggests that the index T_{max} may be a little

Table III. Pharmacokinetic Analysis of the Administered Lorazepam Formulations

Formulation	LS	LP	MP1	MP2
Administration	Oral	Nasal	Nasal	Nasal
T_{max} (min)	25.8 ± 16.9	81.7 ± 45.8	14.2 ± 4.9	9.3 ± 3.8
C_{max} (ng/ml)	6.50 ± 3.72	7.16 ± 2.06	5.21 ± 3.68	6.51 ± 2.88
AUC_{inf} (ng min ml^{-1} , $\times 10^2$)	9.96 ± 2.84	26.48 ± 4.78	5.87 ± 1.07	18.93 ± 3.57
AUC_{20} (ng min ml^{-1} , $\times 10^2$)	0.82 ± 0.38	0.93 ± 0.29	0.72 ± 0.27	1.48 ± 0.41

Data are presented as mean $\pm$ standard deviation ($n=6$)

C_{max} highest drug concentration achieved in the plasma, T_{max} time required to achieve the peak plasma concentration, AUC_{inf} area under the total plasma concentration time curve, AUC_{20} area under the first 20 min of the plasma concentration time curve, *MP1* and *MP2* the two types of spray-dried drug microparticles with vinyl polymer coatings, *LS* lorazepam aqueous suspension, *LP* pure lorazepam particles

Table IV. Relative Bioavailability of the Lorazepam Formulations (a/b)

	LS _b	MP1 _b	MP2 _b	LP _b
LS _a	–	1.69	0.53	0.38
MP1 _a	0.59	–	0.31	0.22
MP2 _a	1.90	3.22	–	0.72
LP _a	2.66	4.51	1.39	–

MP1 and MP2 the two types of spray-dried drug microparticles with vinyl polymer coatings, LS lorazepam aqueous suspension, LP pure lorazepam particles

misleading. The biphasic nature of the PK profile of LP suggested that a proportion of the uncoated lorazepam dose was being absorbed from the pure drug system in almost an equivalent time frame to the vinyl polymer-coated particles. Whilst it may be considered problematic to assign the underlying cause of this biphasic pattern simply from the PK profile, linking it to the aggregate of evidence that has been collected *in vitro*, comparing it to the oral profile and cross referencing to previously published work would seem to suggest that the two spikes in the profile are from sequential IN and oral absorption (14). The timing of the spikes in the PK profile, the small and relatively monodisperse particle size of the lorazepam particles, the dissolution behaviour and the alignment with the reported speed of respiratory mucociliary clearance all support this conclusion.

The MP2 profile was also biphasic with its second spike appearing 70.0 ± 24.5 min after formulation administration. Again, this could be interpreted as a second phase of absorption which was a consequence of the IN administered material being cleared from the nose into the gastrointestinal tract, being dissolved and absorbed; however, analysis of the *in vitro* and *in vivo* data suggests this probably is not the most likely explanation. The rapidity of the vinyl polymer-coated material dissolution and the dissimilarity of the MP2 and LP PK profiles suggest that the biphasic nature of the MP2 may be linked to local mucoadhesion. If this is the case, this explains the superior bioavailability of the MP2 formulation over MP1. *In vitro* data have shown that PVP plasticizes the PVA polymer (22). PVP disrupts the 'crystalline' regions in the PVA by breaking intramolecular bonding, and this activates the capacity of the PVA polymer to form intermolecular hydrogen bonds when it disperses in polar solvents (21). This effect is known to be concentration-dependent, and hence, the MP2 particles that contain a higher ratio of PVP were more likely to facilitate adherence of the lorazepam to the mucus and provide more prolonged drug absorption. The MP1 particles should also dissolve to form a hydrophilic drug-loaded matrix, and this is the reason why it minimised secondary oral absorption, but it does not have the same capacity to 'reach out' and adhere to the mucus through its weaker capability to form hydrogen bonds. It is also possible that the biphasic MP2 profile could be related to the enterohepatic circulation after secretion of lorazepam glucuronide via the bile. However, the time scales involved in oral absorption and enterohepatic circulation post-IN dosing

suggest that it is more likely that these effects manifest in the PK profile beyond the first 100 min. Such effects could be used to explain the slow return to baseline upon oral administration of the drug, *i.e.* in the 200–300-min region of the PK profile. Activated charcoal has been shown to block both oral lorazepam and its glucuronide (33), and the use of activated charcoal prior to intranasal administration of vinyl polymer-coated lorazepam formulations could confirm this interpretation of the data.

At clinically relevant concentrations, about 90% lorazepam is thought to be bound to plasma proteins, and it is the unbound lorazepam that penetrates the blood/brain barrier freely by passive diffusion (24). Therefore, the pharmacokinetic analysis of compounds delivered by the intranasal route must also consider direct entry into the brain tissue and cerebrospinal fluid (CSF) via the olfactory region of the nose (34). Although experimental limitations prevented determination of lorazepam in the CSF during this study, local absorption effects may provide an explanation of the AUC data. Compared to LP, the lower AUC_{inf} for MP1 and MP2 may have been a result of partial drug release by the microparticles when in the nasal mucus, but it also could be a consequence of direct absorption via the olfactory epithelium to the brain and CSF. Direct analysis of the drug levels in CSF is technically challenging, but useful biomarkers of clinical end points are continuing to be developed (35). More data are required to validate biomarkers in animal models and their appropriateness to help interpret effects of agents that act in the CNS, but once this has been achieved, this can potentially aid the understanding of intranasal lorazepam absorption.

CONCLUSION

Intranasally administered lorazepam-rich microparticles achieved a rapid systemic absorption when administered intranasally to rabbits. This provides a clinically relevant treatment option for patients that require a rapid onset of action. The biphasic PK profile obtained when lorazepam was administered as a pure powder coated with almost equal quantities of PVA and PVP demonstrated that the retention of the formulation in the nasal cavity was critical to obtaining a rapid onset of action without secondary oral absorption effects. A single-site absorption profile may be advantageous for lorazepam because this has the potential to provide effective clinical therapy without residual sedation in patients (36).

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