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Research Article

Cytarabine Pod Infusion Bolus in Dogs with Meningoencephalitis of Unknown Etiology

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Abstract

This study evaluated the pharmacokinetics (PK) of an infusion bolus (IB) of cytarabine (CA) administered subcutaneously (SC) via a novel delivery system (Omnipod®) to dogs with meningoencephalitis of unknown etiology (MUE). Dogs with MUE were enrolled at NC State Veterinary Hospital and received a 75mg/m2 IB SC dose of cytarabine via Omnipod® over 60 minutes. Six dogs were entered into the study, and a total of ten bioavailability profiles were collected. The study design was prospective and non-randomized and used a sparse sampling technique. Plasma CA concentrations were measured by high-pressure liquid chromatography. The mean plasma concentration (Cmax) was 3578 ± 752 ng/mL, the average time to Cmax (Tmax) was 78.89 ± 6.44 minutes. Nonlinear mixed effects modeling was used to obtain population estimates for the absorption rate constant (Ka), clearance per fraction absorbed (Cl/F), and volume of distribution per fraction absorbed (V/F). The mean plasma concentration of CA for all measured time points was above 1000 ng/ml at the 30, 60, 90, 105, 120, 150, 180 and 240-minute time points. Following a single SC IB of 75 mg/m2 of CA in dogs with MUE, the PK of CA was similar to values previously reported in healthy beagles and dogs with MUE when administered via intravenous (IV) and subcutaneous (SC) routes. Therefore, Omnipod® IB may be an effective alternative to CA's traditional injection protocols.

Keywords: Dog; MUE; Cytarabine; Pod; Bolus

Introduction

Meningoencephalitis of unknown etiology (MUE) is among the most common causes of inflammation in the central nervous system of dogs [1]. Cytarabine (CA) and glucocorticoids are widely used to treat dogs with MUE [2-6]. Cytarabine is an antimetabolite therapeutic pyrimidine analog known as arabinosylcytosine (ARA-C) [17]. Once inside the cell, it is converted to a triphosphate, competing with cytidine for incorporation into DNA [17]. Cytarabine inhibits DNA replication and repair by inhibiting DNA polymerase during the S phase of the cell cycle [17]. An advantage of this medication is that it is highly bioavailable when administered subcutaneously, intrathecally, or intravenously with a high volume of distribution. Cytarabine can also readily cross the blood-brain barrier [17]. Metabolism of CA occurs primarily in the liver, and the kidneys eliminate its metabolites [17].

There are various CA administration protocols via subcutaneous (SC) and intravenous (IV) routes. Previous pharmacokinetic studies have demonstrated a clinical utility with values greater than 1 µg/mL (1000 ng/mL) after administration of CA via both

routes [9,14,15]. A study by Lowrie., *et al*. demonstrated a significant improvement in three-month survival rates for dogs that received CA via a constant rate infusion (CRI) (100 mg/m^2 over 24 hours (h)) for initial treatment when compared to dogs receiving SC administration (50 mg/m^2 SC q12 h for two doses) [7]. There are disadvantages to IV administration, including higher expenses, lengthier hospital stays, and repeated IV catheterization, making IV access increasingly difficult. It was recently demonstrated that a continuous subcutaneous infusion over 8 hours with the Omnipod® system resulted in similar bioavailability comparable to other IV CRI or SC dosing protocols [8-1]. This is the second study to evaluate CA delivery with the Omnipod® system and to establish its use as a practical alternative for CA delivery. This study aimed to assess an SC infusion bolus (IB) of CA for 75mg/m2 delivery over 60 minutes using the Omnipod® delivery system. It was hypothesized that the CA pharmacokinetics of an SC IB would be similar to traditional SC injections.

Material and Methods

Patient enrollment

Six client-owned dogs diagnosed with MUE were enrolled at NC State University Veterinary Hospital (NCSU VH). The NCSU Hospital Board and the NCSU Institutional Animal Care and Use Committee approved this study (Amended Protocol 20-140). Dogs were excluded if they weighed more than 10 kg, as the pod infusion system accommodates a maximum drug volume of 2.00 ml. Bioavailability profiles were assigned to two groups without randomization in an alternating pattern based on their scheduled clinic appointments. Group one had samples collected at the time points: 30, 90, 120, and 180 minutes. Group two had samples collected at the time points: 60, 105, 150 and 240 minutes. Four dogs had samples collected in more than one group, with a total of 10 bioavailability profiles obtained. Table 2 below outlines the sparse sampling technique used.

Cytarabine administration

Cytarabine was administered via a commercially available SC delivery system, Omnipod®, typically used for human insulin delivery. The Omnipod® can be programmed to deliver infusions for up to 72 hours and/or intermittent IB. The maximum bolus delivery rate possible with the Omnipod® was 0.01 ml/40 seconds, with a basal delivery rate ranging from 0.0005 to 0.3 ml/ hr. A standard duration and rate were used as the IB such that the delivery could be performed at the maximum system basal rate, which took into account the differences in dog size in the study design. The Omnipod DASH® insulin management system consists of the Omnipod® delivery system and the personal diabetes manager (PDM), a Bluetooth device similar to a smartphone. In this study, the Omnipod® system was programmed to deliver CA as a 75mg/m^2 SC IB over 60 minutes.

The CA dose was prepared and drawn into a 3ml Luer-lock syringe by the NCSU Pharmacy in a containment hood while the pharmacist wore chemotherapy-rated personal protective equipment. A state-of-the-art closed-system transfer device from EQUASHIELD® was attached to the syringe to prevent any escape of CA. The investigator wore nitrile gloves and a face shield to load the CA into the pod. An approximately 7 x 10 cm region on the patient's dorsum, just caudal to the scapulae, was clipped and wiped with alcohol. The Omnipod® was applied directly to the patient's skin. A needle and cannula are inserted underneath the skin when the system is programmed via the blue-tooth device. A PodPal®, an extra adhesive bandage designed for use with the OmniPod®, was applied around the pod. Finally, a vest, typically used for remote cardiac monitoring, was placed on the patient over the Omnipod® to further secure the system. A PDM was used to deliver CA as an IB at a rate of 75 mg/m^2 over 60 minutes. At the completion of the study, the PodPal® and Omnipod® were removed using Medi-Sol®, an adhesive remover. All materials, including pods and gloves, were disposed of as hazardous chemotherapy waste.

Sample collection

A sparse sampling technique was used as it minimizes the volume of blood obtained from small dogs [12]. As mentioned, the dogs were divided into two groups. In group one, blood samples were drawn at 30, 90, 120, and 180 minutes, and in group two, blood samples were drawn at 60, 105, 150, and 240 minutes. See table 2 for a representation of this sparse sampling technique. A zero-time point was not obtained to decrease the number of IV punctures in these patients. Blood samples (1.2ml) were collected via direct jugular venipuncture and placed in lithium heparin tubes. The blood samples were centrifuged immediately at 1,380 g for 10 min. The plasma was harvested and frozen at -80°C until analysis; storage time varied from 2 weeks to 6 months. Each dog received an additional 225 mg/m2 of CA as a CSCI over 4 h via the Omnipod® as part of their scheduled MUE treatment after the last blood sample was drawn. Plasma samples were analyzed using high-pressure liquid chromatography-tandem mass spectrometry (HPLC-MS) [14,15]. with the 6500+ QTRAP LC-MS/MS system (Sciex, Framingham, MA) in the Duke Proteomics and Metabolomics Shared Resource, Center for Genomic and Computational Biology, Duke University School of Medicine, Durham, NC. Software Analyst 1.7.1 was used for data acquisition and analysis. All data was analyzed in the software Skyline (daily version 22.2.1.278). It includes raw data import, peak integration, and the linear regression fit with 1/x weighting for the calibration curve.

Pharmacokinetics

Pharmacokinetic modeling was conducted using commercially available software (Phoenix NLME version 8.3.5, Certara, Princeton, NJ). Using a nonlinear mixed-effects approach, PK parameter estimates were generated. Different base models were assessed for fit based on visual inspection of the plasma concentration vs. time data, the goodness of fit and residuals plots, and Akaike's Information Criteria; the final model was a one-compartment extravascular model with a lag-time and first-order absorption and elimination, parameterized by clearance. Random effects for Ka was removed from the model due to the high shrinkage (>0.8) associated with this value. Covariate tests on the parameters were not performed. Relative variability was described using a multiplicative error term. Final model validation was performed using a bootstrap method on 1,000 replicate data sets, and visual predictive checks were used to evaluate the final model further. Secondary PK parameters (the elimination rate constant (Ke), absorption, and elimination half-lives) were determined using standard PK equations.[13] The mean plasma concentration (Cmax), time to maximum plasma concentration (Tmax), and were determined directly from the data. All data are presented in the form of descriptive statistics.

Results

A total of six client-owned dogs diagnosed with MUE were enrolled in the study. From these dogs, a total of 10 plasma profiles were created via a sparse sampling technique. The age of the dogs ranged from 1.2 to 7.9 years old (median 4.5 years). The weight of the dogs ranged from 3.45 to 9.70 kg (median 5.02 kg). There were two spayed females, one intact male, and three neutered males. The following breeds were represented in this population: French Bulldog (2), Chihuahua, Shih Tzu, Dachshund, Pomeranian, and Terrier mix. All six dogs enrolled were treated with daily prednisone at doses varying from 0.21-1.75 mg/kg/day. One dog also received leflunomide at a dose of 2 mg/kg/day for adjunct treatment of their MUE.

The PK analysis used 40 plasma samples. Plasma drug concentrations ranged between 267 and 5818 ng/ml. The mean Cmax was 3578 ± 752 ng/mL, with a mean Tmax of 78.89 ± 6.44 minutes. Results are summarized below in Figure 1 and Table 1. The mean Cmax for each dog was above 1000 ng/mL at the 30, 60, 90, 105, 120, 150, 180, and 240-minute time points.

No serious adverse effects were reported by the owners following CA administration via Omnipod®. Two dogs had a mild, 1 x 1 mm erythematous skin lesion at the needle and cannula insertion site, which resolved within 24 hours of hospital discharge.

Parameter	Mean or TV	SD or CV%
Cmax (ng/mL)	3578	752
Tmax (min)	78.89	6.44
Ka (1/min)[a]	0.014	n/a
Vd/F (mL/kg)	280.2	44.6%
Cl/F (mL/kg*min)	7.7	33.7%
Tlag (min) [a]	23.8	n/a
Residual error (5)	22.3	n/a

Table 1: Cytarabine plasma pharmacokinetic values in six dogs administered 75mg/m^2 via SC infusion bolus using an Omnipod® system. TV: population estimate; CV%: coefficient of variation/inter-individual variability; Ka: Absorption rate constant; Vd/F: Volume of distribution per fraction absorbed; Cl/F: clearance per fraction absorbed; Tlag: Lag time. n/a: Not applicable

[a]Interindividual variability is not calculated because of excessive shrinkage (>0.4).

	30 min	60 min	90 min	105 min	120 min	150 min	180 min	240 min
Patient 1a	X		X		X		X	
Patient 1b		X		X		X		X
Patient 2a	X		X		X		X	
Patient 2b		X		X		X		X
Patient3a	X		X		X		X	
Patient 3b		X		X		X		X
Patient 4a	X		X		X		X	
Patient 4b		X		X		X		X
Patient 5a	X		X		X		X	
Patient 6a		X		X		X		X

Table 2: Sparse sampling technique used with associated time points of blood collection for each patient. Patients were numbered 1-6. The letters a and b refer to the time schedule for blood collection. Schedule a had samples collected at 30, 90, 120, and 180-minute time points during a single day. Schedule b had samples collected at the 60, 105, 150, and 240-minute time points during a single day.

Discussion

This study investigated a novel device for delivering an SC IB of CA to six dogs with MUE. A total of 10 bioavailability profiles were collected. The mean plasma concentration for each dog was above 1000 ng/mL at all the sampled time points. Although therapeutic levels of CA have yet to be established, other studies have cited 100 ng/mL as a minimum therapeutic level [10], it should be noted that all dogs in the present study had CA levels above 100 ng/mL at the last sampling time point (250 min).

Cytarabine delivery via SC IB showed comparable pharmacokinetic values to a single SC injection of CA, despite different clinical populations, doses, routes, and PK analysis methods (NLME versus NCA versus compartmental methods), in particular for clearance or clearance per fraction absorbed [9-11]. Results were also comparable to the pharmacokinetic parameters seen in healthy beagle dogs receiving CA via IV and SC routes, although the estimated elimination half-life in the present study was shorter at approximately 25 minutes.[9] The Omnipod® offers a reasonable option of providing programmable intermittent IB and CRI as an alternative method of CA administration.

The 60-minute SC IB of CA was evaluated because of the many differences in clinical management protocols and that in the previous study (Mancini., *et al*. 2022), there were no time points/blood samples collected before 4 hours. The 60-minute infusion bolus was chosen as it allowed the investigators to evaluate the pharmacokinetics parameters of a single bolus dose over 3-4 hours at different time points. A limitation of this study was the duration of sampling, as these were client-owned dogs who needed to receive the full cytarabine dose before discharge from the hospital the same day. Study enrollment was restricted to dogs less than 10 kg because the Omnipod® reservoir has a maximum capacity of 2.00 mL, limiting overall drug delivery. This limitation is not expected to alter the pharmacokinetic findings but may limit the utility of the Omnipod® in larger patients depending on the dose of CA given. Another possible limitation of Omnipod® use is that it can only bolus at a rate of 0.01ml per 40 seconds. An additional concern is that the Omnipod® may not remain patent or may be inadvertently removed (via rough handling, patient activity, or by another dog). This could result in the spilling of CA or ingesting the Omnipod® itself, both of which can be harmful. This risk was minimized by placing a vest, cage confinement, and monitor activity. Although this risk was minimized in the hospital setting, this could be a concern for future outpatient applications.

This study used a sparse sampling technique because all patients were small-breed dogs, which allowed us to limit the number of blood draws for each patient. Sparse sampling also limited the number of venipunctures per animal and likely reduced iatrogenic trauma and subsequent difficulty with venipuncture. This method is often used in PK studies in small laboratory animals for similar reasons. Previous research comparing sparse sampling to serial sampling in rodent toxicokinetic studies has shown that proper study design can yield PK objectives while minimizing the amount of blood drawn per animal [16]. This is a useful clinical technique as it allows sampling in our current patient population and is not expected to alter the PK findings.

Conclusion

This study found that an SC IB delivery of CA using the Omnipod® system yielded PK parameters similar to those obtained with injection administration via the IV or SC routes currently used. Thus, SC IB delivery via Omnipod® may be an effective alternative to traditional CA injection protocols. Further studies are needed to evaluate the feasibility of this delivery system for outpatient treatment of dogs with MUE with cytarabine.

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Funding and Conflict of Interest Statement

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Authors Contribution

- PJE, SLM, SLF designed the research, formulated the plans, and supervised the experiment.
- PJE, SLM, SLF, NJO, CLM, KMM assisted in data collection and analysis.
- PJE, SLM, SLF, KMM, NJO, CLM, KRM, XL, and BMS reviewed and edited the manuscript.

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