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# The bioavailability of cytarabine in dogs with meningoencephalitis of unknown etiology through iontophoresis and rectal delivery

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**Abstract**
**Background:** Cytarabine (CA) is used to treat dogs with meningoencephalitis of unknown etiology (MUE) by subcutaneous or intravenous administration.
**Aim:** The objective was to investigate transdermal iontophoresis and rectal administration as alternative routes of CA delivery.
**Methods:** Two client-owned dogs with MUE were studied. The ActivaPatch® IONTOGO™ 12.0 iontophoresis drug delivery system delivered 200 mg/m2 CA transdermally. Blood samples were collected by sparse sampling technique after initiation of the device. At another visit, 100 mg/m2 CA was administered rectally. Blood samples were collected by sparse sampling technique after administration. Plasma CA concentrations were measured by high-pressure liquid chromatography.
**Results:** The concentration of plasma CA after transdermal and rectal administration was below the limits of quantification (0.1 μg/ml) in all samples suggesting inadequate bioavailability with transdermal and rectal administration.
**Conclusion:** Transdermal and rectal CA administration are not reasonable alternative routes of delivery.
**Keywords:** Dog, Meningoencephalitis, Cytarabine, Bioavailability.

## Introduction

Meningoencephalitis of unknown etiology (MUE) is a term that refers to a variety of non-infectious inflammatory diseases of the central nervous system. Currently, there is no standard treatment protocol, but many studies have shown that glucocorticoids combined with a second immunosuppressive agent can yield successful outcomes (Zarfoss *et al*., 2006; Coates *et al*., 2007; Lowrie *et al*., 2016).

Cytarabine (CA) has gained growing popularity in the treatment of immune-mediated diseases. Subcutaneous (SC) and intravenous (IV) routes of CA administration have been described in dogs (Crook *et al*., 2012; Pastina *et al*., 2018). However, other routes of CA administration have not been well investigated to determine if there are reasonable alternatives for effective delivery.

Transdermal iontophoresis applies an external electrical potential difference enabling the movement of ions across a membrane. Transdermal drug administration is a non-invasive yet systemic delivery system that avoids hepatic first-pass metabolism and eliminates gastrointestinal absorption. Iontophoresis is a promising modification for transdermal drug delivery that provides reliable, programmed drug delivery while avoiding physicochemical factors that interfere with adequate absorption (Dixit *et al*., 2007; N'Da, 2014).

Rectal administration has been utilized as a route of medication delivery for many years and has even been shown to exceed oral absorption and bioavailability by at least partial avoidance of hepatic first-pass metabolism (De Leede *et al*., 1984; Papich and Acorn, 1995). These findings warrant further investigation into rectal delivery of CA in cases of canine MUE.

The purpose of this study was to measure plasma concentrations of CA after transdermal iontophoresis and rectal administration in two canine patients diagnosed with MUE to identify alternate routes of administration.

## Materials and Methods

Two client-owned dogs diagnosed with MUE were evaluated in this study; A ten-year-old male neutered Chihuahua and a four-year-old female spayed Labrador Retriever. The dogs were enrolled through the NC State University Veterinary Hospital. The diagnosis of MUE was based on clinical signs, magnetic resonance imaging findings, and results of CSF analysis (Granger *et al*., 2010). Free access to water was provided throughout the study. The dogs were fasted from the evening before the study, for a minimum of 8 hours before CA administration, until the study was completed.

### *Iontophoresis administration*

Transdermal administration was investigated in two dogs. The ActivaPatch® IONTOGO™ 12.0 is a self-powered, disposable, single-use non-invasive iontophoresis drug delivery system that was used to

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deliver CA transdermally by use of an 80 mAmP*min dose over 12 hours. Prior to application, the dorsal aspect of the dog's neck was shaved. The skin was cleaned with an alcohol wipe, then air-dried. The ActivaPatch® IONTOGO™ 12.0 was prepared according to the manufacturer's directions. CA was drawn up into a syringe as a 200 mg/m$^2$ dose formulated to 2 ml with normal saline to fit within the ActivaPatch® IONTOGO™ 12.0 2 ml reservoir. Using the syringe, the CA was added to the reservoir to hydrate the active ionic solution reservoir. The outer liner was removed, and the patch was placed over the shaved treatment area. The patch was removed, and discarded 12 hours (720 minutes) after application, once treatment was completed as indicated by the LED indicator light.

Blood samples (2-3 ml) were collected using a sparse sampling technique via jugular or saphenous venipuncture with a 20-22 gauge needle. Sparse sampling allows fewer blood samples to be taken from a patient during the study. These samples are then pooled from different patients to generate a bioavailability profile. Blood samples were collected at 0, 120, 360, 600, and 840 minutes after the application of the ActivaPatch® IONTOGO™ 12.0 in the first dog and in the second dog, samples were collected at 0, 240, 480, 720, and 960 minutes after the application of the ActivaPatch® IONTOGO™ 12.0.

***Rectal administration***

Rectal administration was investigated in one dog, the ten-year-old male neutered Chihuahua, at two separate visits to the NCSU VH, 3 weeks apart. Prior to administration, the rectum was digitally evacuated, and the dog was subsequently taken outside for 5 minutes to allow for voluntary defecation. Afterward, the rectum was once again digitally evacuated. A 100 mg/m$^2$ dose of CA was measured into a 1-ml syringe. Lubricant was applied to the sides of the syringe tip, and the syringe was inserted 3 cm into the rectum. The CA was delivered rectally in this manner, and then the rectum was held closed for 2 minutes with light manual pressure.

Blood samples (2-3 ml) were collected in the same manner as described for collection after transdermal administration (sparse sampling technique) at 0, 30, 90, and 240 minutes after the initial administration of rectal CA and blood samples were collected at 0, 60, 120, and 360 minutes after administration of rectal CA at the second treatment 3 weeks later.

***Sample handling***

Samples were placed in lithium heparin tubes, spun, and plasma was collected. Plasma samples were transferred and preserved in cryogenic tubes at −80°F until the time of analysis by high-pressure liquid chromatography (HPLC). Plasma samples were each analyzed by HPLC using a method developed and validated in the NCSU-CVM Clinical Pharmacology Laboratory in a previous study (Crook *et al*., 2012).

***Treatment after sample collection***

After all blood samples were collected, cytarabine was delivered to the patient as a dose of 75 mg/m$^2$ SC every 2 hours for four total doses to compensate for the potential for poor absorption and bioavailability. Therefore, at minimum 300 mg/m$^2$ was administered, and at maximum 400 or 500 mg/m$^2$ was administered for rectal and transdermal iontophoresis delivery, respectively.

***Ethics approval***

This study was approved by the NCSU-CVM Institutional Animal Care and Use Committee (Protocol 15-088-0).

## Results and Discussion

There were no obvious adverse effects or complications observed with rectal or transdermal administration of CA. The concentration of plasma CA after transdermal administration of a dose of 200 mg/m$^2$, was below the limits of quantification (0.1 µg/ml) in all samples. The concentration of plasma CA after rectal administration of a dose of 100 mg/m$^2$, was below the limits of quantification (0.1 µg/ml) in all samples.

These results compare to a peak concentration ($C_{max}$) of 2.88 +/− 0.3 µg/ml or 2.8 +/− 0.39 µg/ml for SC (single SC injection of 50 mg/m$^2$) and constant rate infusion (CRI) 25 mg/m2/hours for 8 hours administration, respectively (Crook *et al*., 2012). Time to reach maximum concentration ($T_{max}$) was 1 hour after SC administration and 4 hours after IV administration (Crook *et al*., 2012). This comparison indicates that transdermal and rectal routes were inadequate for effective CA delivery in these two cases compared to the current SC and IV standards.

Transdermal administration was evaluated as one alternative route of delivery. Iontophoresis applies an external electrical potential difference to enable the movement of ions across a membrane (Dixit *et al*., 2007). CA, specifically, has a low partition coefficient, meaning that it is too hydrophilic to pass into the stratum corneum (N'Da, 2014). It was the expectation of this study that iontophoresis would improve the transdermal bioavailability of CA. Iontophoresis also theoretically mimics the dynamics of a CRI, which has been proven superior to bolus SC injections at maintaining CA at a steady-state within plasma in canine patients (Crook *et al*., 2012). The dose of CA was 200 mg/m$^2$ for the iontophoretic trials. There was no detectable absorption of CA in the two canine patients at any time point throughout the study. An inherent limitation of a transdermal drug delivery patch exists in its ability to hold a specific volume so the dose that is physically capable of being utilized with a patch becomes a limiting factor. This restricts future studies investigating iontophoresis bioavailability with higher doses.

Rectal administration was a promising alternative route of delivery because substances absorbed by the lower rectum are drained by the inferior and middle rectal veins, which are directly connected to the systemic circulation and thereby avoid hepatic first-pass metabolism (De Leede *et al*., 1984; Papich and Alcorn, 1995; Evans and deLahunta, 2013). The dose of CA for rectal administration was 100 mg/m$^2$. Still, such behavior of absorption was not recognized in this study as there were no measurable plasma levels of CA after rectal administration. Another study evaluated rectal CA administration in rats utilizing the use of an adjuvant, palmitoyl-DL-carnitine, which increased the initially poor bioavailability of CA to approximately 9% with (Fix *et al*., 1986). Further studies are warranted to determine whether adjuvants could be utilized to maximize intestinal absorption of CA in canine patients. This study was limited by the small number of cases enrolled and non-standardized sampling times. Initially, this study was planned as two studies investigating transdermal and rectal CA delivery separately. The sampling times were standardized in each individual study, but not across both studies. Furthermore, as the initial results indicated such poor bioavailability of CA, each study was discontinued before completion. The results that were collected were combined in this short communication to share the negative results.

## Conclusion

This study investigated innovative routes of CA delivery in two canine patients diagnosed with MUE. It determined that there was no measurable plasma level of CA, up to 6 hours after rectal administration. There was also no measurable plasma level of CA up to 16 hours after transdermal administration with the ActivaPatch® IONTOGO™ 12.0 in these cases. These alternative routes of CA administration are not considered reasonable alternative routes of delivery at this time.

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***Conflict of interest***

The authors declare that there is no conflict of interest.

***Authors' contribution***

P.J.E designed the research, formulated the plans, and supervised the experiment. P.J.E., B.O.P, N.J.O, C.L.M., and K.R.M assisted in data collection. S.L.M., P.J.E., N.J.O, C.L.M., K.R.M, and B.O.P. wrote and reviewed the manuscript. All authors critically reviewed the data and the manuscript.

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