# Effect of a constant rate infusion of cytosine arabinoside on mortality in dogs with meningoencephalitis of unknown origin

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## ARTICLE INFO

## ABSTRACT

Administration of cytosine arabinoside (CA) by continuous rate infusion (CRI) has pharmacokinetic and pharmacodynamic advantages over traditional intermittent dosing. Whether these advantages translate into clinical efficacy remains unknown. The aim of this study was to assess the efficacy and safety of CRI of CA in dogs with meningoencephalitis of unknown origin (MUO) and to compare outcomes with a group of historical control dogs treated with conventional intermittent subcutaneous (SC) administration of CA; both groups received adjunctive prednisolone. It was hypothesised that a CRI of CA for 24 h at 100 mg/m$^2$ would improve survival and lesion resolution compared with conventional SC delivery of 50 mg/m$^2$ every 12 h for 48 h. Eighty dogs with suspected MUO were recruited from consecutive dogs presenting with suspected MUO from 2006 to 2015. All dogs underwent routine clinical evaluation, magnetic resonance imaging of the brain and cerebrospinal fluid analysis. There were 39 dogs in the SC group and 41 dogs in the CRI group; baseline characteristics were similar in both groups. Survival at 3 months was 22/39 (44%) with SC delivery versus 37/41 (90%) with CRI. No dose-limiting toxicities were noted for either group. The resolution rate of magnetic resonance imaging and cerebrospinal fluid abnormalities at the 3 month re-examination were substantially improved in the CRI group versus the SC group. The CRI regimen produced a survival advantage over the SC route of administration without clinically significant toxicity. These data supports the routine use of CRI at first presentation for the treatment of MUO in dogs.

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## Introduction

Meningoencephalitis of unknown origin (MUO) presents a diagnostic and therapeutic challenge for the veterinary clinician. A gold standard diagnosis is achieved by obtaining material for histopathology, although the inaccessibility of the affected tissue increases the morbidity and mortality if performed as an antemortem procedure (Koblik et al., 1999; Flegel et al., 2012; Rossmeisl et al., 2015). In most cases, a presumptive diagnosis is reached on the basis of neurological examination, cross-sectional imaging findings, cerebrospinal fluid (CSF) analysis and negative titres for selected infectious agents, which together indicate inflammation without infectious disease (Granger et al., 2010).

In the past 2 decades, a substantial body of work has been published concerning the outcome of various immunosuppressive therapies for MUO. In spite of the large number of studies, there remains considerable controversy about optimal treatment and there is currently no universally accepted standard of care. This is due to the variability in outcome resulting from a heterogeneous disease process requiring large sample sizes to give statistically meaningful conclusions. Many of the published studies have failings; studies tend to enrol relatively few animals, inclusion criteria are variable and obtaining meaningful mortality data is hampered by long survival times in many cases. This leads to loss of cases to follow-up and increases the likelihood that survival might be influenced by factors other than disease progress, such as comorbidities and financial considerations. Moreover, some studies exclude dogs that die shortly after admission, both before and after instigation of treatment (Zarfoss et al., 2006; Menaut et al., 2008). Since mortality in MUO is common in the first 72 h (Muñana and Luttgen, 1998; Lowrie et al., 2013), exclusion of this subset of dogs gives a false impression of the efficacy of any medication.

Most authors agree that prednisolone is the mainstay of therapy for MUO (Granger et al., 2010; Talarico and Schatzberg, 2010). Controversy arises when considering if there is need for an adjunct immunosuppressive medication and, if administered, which medication. Cytosine arabinoside (CA) is one of the more commonly prescribed adjunctive treatments. This drug acts as an intercalating agent, targeting rapidly dividing cells, and is commonly used as a chemotherapeutic agent in lymphosarcoma (lymphoma) and as an immunosuppressant agent.

We and others have previously published our observations of the outcome in MUO when using prednisolone combined with subcutaneous (SC) CA (Zarfoss et al., 2006; Menaut et al., 2008; Smith et al., 2009; Lowrie et al., 2013). The SC route was originally chosen, as it was previously considered that this route would produce a slow and prolonged absorption of the drug, which is necessary to maximise the effect of CA on rapidly dividing cells (Cozzarelli, 1977). However, pharmacokinetic data reveal rapid absorption, but with a similarly rapid decrement in concentration when administered subcutaneously, whereas intravenous constant rate infusion (CRI) produces a sustained plasma concentration during the time course over which it is administered (Scott-Moncrieff et al., 1991; Crook et al., 2013).

In the current study, we investigated the safety and efficacy of a CRI of CA administered over 24 h and compared mortality at 3 months with that achieved in historical control cases using a conventional 2-day SC dosing regimen. Since we have previously shown that those dogs surviving the initial stages of disease typically achieve long term survival (Smith et al., 2009; Lowrie et al., 2013), we focused on the mortality rate at 3 months following diagnosis, with follow up magnetic resonance imaging (MRI) and CSF analysis at this time recorded as a secondary outcome.

## Materials and methods

### Dogs

This study adds to the data from a previous prospective treatment trial of the effect of prednisolone and CA administered subcutaneously to dogs with presumptive MUO. Details of this study are described elsewhere (Lowrie et al., 2013). Dogs with presumptive MUO presented consecutively to the small animal neurology service at Davies Veterinary Specialists from May 2006 to August 2015 were recruited prospectively. Dogs with a history of steroid administration prior to presentation were excluded from the study. Signalment, history, physical and neurological examination were recorded, including the duration of clinical signs before investigation. A minimum database for each dog consisted of complete blood count (CBC), serum biochemistry profile, serum antibody titres to *Neospora caninum* and *Toxoplasma gondii* (assayed by indirect fluorescence antibody tests), MRI of the brain, and CSF analysis (cytology and total protein concentration). MRI examinations were performed using a 0.4 T magnet. Pulse sequences varied, but always included sagittal and transverse T2-weighted images (T2-WI); transverse T2-fluid-attenuated inversion recovery (FLAIR) images; and transverse T1-weighted images (T1-WI) before and after paramagnetic contrast injection, including a subtraction manoeuvre to highlight regions of contrast enhancement. All dogs had to have at least a 3 month follow-up after initiating therapy and all dogs that died within this time were included in the survival analysis.

### Diagnosis

A presumptive diagnosis of MUO was based on guidelines from a previous study (Granger et al., 2010). Dogs were considered to have MUO if they were older than 6 months, with evidence of single, multiple or diffuse intracranial lesions on MRI, CSF pleocytosis (total nucleated cell count, TNCC >5 nucleated cells/µL; erythrocyte count <4000 cells/µL; >50% mononuclear cells and an absence of antibodies against *Neospora caninum* and *Toxoplasma gondii*. Dogs with focal cortical lesions that appeared hypointense on T1-WI were excluded from the study (given this may represent necrotising encephalitis, a more aggressive variant of inflammatory CNS disease), as were those with the optic form of granulomatous meningoencephalitis (GME, i.e. those dogs with inflammation of the optic nerve but with no lesions in the brain parenchyma). The presence or absence of eight MRI characteristics was determined (Table 1).

### Treatment

All dogs were treated with a standard protocol commencing with immunosuppressive doses of prednisolone and CA in accordance with guidelines from other studies (Zarfoss et al., 2006; Menaut et al., 2008). Prednisolone was administered as per Fig. 1 and was identical in both groups. The first 39 dogs were administered subcutaneous CA at a dose of 50 mg/m$^2$ every 12 h for 2 days; the outcome in this group has been reported previously (SC group; Lowrie et al., 2013). Subsequent dogs were administered CA as a continuous rate infusion at a dose of 100 mg/m$^2$ over 24 h (CRI group). Following this initial treatment, both groups received subsequent CA in the same manner as the SC group (i.e. subcutaneous administration at a dose of 50 mg/m$^2$ every 12 h over 2 days (Fig. 1), initially at three weekly inter-

vals. A CBC was collected 3 weeks following each administration of CA and the owners were asked if adverse effects had been observed.

### Outcome

The primary outcome was mortality at 3 months. All dogs that died or were euthanased were recorded and survival was compared as a binary variable with the group of historical control dogs (i.e. those given subcutaneous CA). Re-examination was scheduled for all surviving dogs 3 months following the start of treatment, at which point MRI scans and CSF analysis were repeated. MRI and CSF findings were classified as normal or abnormal at this time (abnormal CSF defined as TNCC >5 white blood cells/µL and/or total protein concentration >25 mg/dL).

### Statistical analysis

Baseline characteristics (age, delay to presentation, sex, CSF nucleated cell count, CSF protein concentration and the eight MRI features listed in Table 1) of dogs in the two groups were compared. For continuous variables, median values were calculated (including ranges) and compared using the Wilcoxon rank sum test, taking $P < 0.05$ as the level of statistical significance. For categorical variables, frequencies were calculated and compared using a $\chi^2$ test, again using $P < 0.05$ as the level of statistical significance. The primary outcome measure was survival at 3 months, which was calculated and analysed using Fisher's exact test. Long term survival analyses were conducted using Kaplan–Meier plots and were compared by log-rank analysis; $P < 0.05$ was considered to be statistically significant. Statistical analysis of the recorded MRI data at first diagnosis from the CRI group was performed to determine if any of the eight MRI features (Table 1) were predictive of survival. The association between each MRI finding and survival was tested using Fisher's exact test with statistical significance set at $P < 0.05$. If an MRI finding had significance, likelihood ratios and confidence intervals were then calculated. Our previous study has reported this same calculation for the SC group (Lowrie et al., 2013).

## Results

A total of 80 dogs were included in the analysis. Of these, 39 (49%) were given subcutaneous CA and acted as historical controls and 41 (51%) were prospectively recruited and given a CRI of CA. There was no difference in age at presentation, sex, delay to presentation or CSF analysis between the two groups (Table 2). We have previously shown that a number of MRI features at diagnosis can have an impact on mortality (Lowrie et al., 2013) and therefore both groups were compared for the frequency of these predictive factors; no significant differences were identified (Table 1).

Mortality (death or euthanasia) at 3 months was 22/39 (56%) in dogs given SC CA and 4/41 (10%) in the CRI group. Log rank analysis of the Kaplan–Meier survival curves for the two groups during this 3 month period confirmed that this represented a significantly better survival for CRI dogs compared to SC dogs (Fig. 2; log-rank test $P < 0.0001$). The proportion of dogs alive at 3 months that survived long-term showed that 37/37 (100%) in the CRI group were still alive at 12 months and 22/22 (100%) in the SC group were still alive at 12 months ($P = 0.824$).

Our secondary outcome measure was the occurrence of MRI and CSF abnormalities at follow-up. Thirty-four of 37 (92%) surviving dogs in the CRI group had a normal MRI scan at 3 months, compared with 7/17 (41%) surviving dogs in the SC group; this difference was statistically significant ($P < 0.001$). In addition, CSF was also

**Table 1**
Summary for qualitative variables of dogs receiving cytosine arabinoside in each group.

| MRI Features at first presentation | Subcutaneous group Yes (%) | CRI group Yes (%) | P value |
|---|---|---|---|
| Single lesion | 12 (31) | 7 (17) | 0.15 |
| Sulci effacement | 18 (46) | 17 (41) | 0.67 |
| Rostral fossa involvement | 25 (64) | 27 (66) | 0.87 |
| Caudal fossa involvement | 24 (62) | 26 (63) | 0.86 |
| Contrast enhancement | 15 (38) | 16 (39) | 0.96 |
| Mass effect | 26 (67) | 33 (80) | 0.16 |
| Foramen magnum herniation | 14 (36) | 18 (44) | 0.58 |
| Transtentorial herniation | 12 (30) | 20 (49) | 0.10 |

CRI, constant rate infusion; MRI, magnetic resonance imaging.