Pharmacokinetics of citicoline after intravenous and intramuscular administration in dogs

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Abstract

Background: New studies have confirmed the role of citicoline in reversing different pathological conditions in canine medicine, but dosing regimens of this drug has not yet been investigated in dogs. Using a high-performance liquid chromatography (HPLC) system, this study aims to quantify the levels of citicoline in dogs plasma after intravenous (IV) and intramuscular (IM) administration.

Design and methods: The subjects of this study were 12 male, mixed breed healthy dogs that were approximately 2 to 3 years old and had a body weight between 15 and 25 kg. Plasma samples were extracted following protein precipitation. Samples were eluted from the column at flow rate of 0.7ml min⁻¹. Solvents were degassed. The PH of the mobile phase of HPLC grade acetonitrile: water (20:80, v/v) was adjusted to 3.0 using 1% orthophosphoric acid. Both sample and standard solutions were filtered through 0.22 µm membrane filter. A sample volume of 20 microliter was injected to HPLC to obtain a standard curve.

Results: No clinical signs or drug adverse effect was noticed in animals during and after the period of administration of citicoline. Measuring plasma concentration of citicoline sodium after IV and IM administration showed biphasic plasma peaks which occurred at 15 minutes and 5 hours after the drug administration in dogs. Conclusions: Giving the drug once a day with 30-50 mg/kg dosage or twice daily with 15-30 mg/kg dosage would cause their levels to remain elevated for much of the day and doesn’t have any serious adverse effect.

Keywords: Citicoline, Intravascular, Intramuscular, Kinetic, Dog

Introduction

The rationale administration of a drug regimen requires basic information of pharmacokinetics in the animal species, and this prepares optimum clinical efficacy.

Citicoline (cytidinediphosphocholine, CDP-choline) is a complex organic mononucleotide composed of ribose, cytosine, pyrophosphate, and choline (1). It is a water soluble compound which is a neuroprotective when administered exogenously. It is also an intermediate in membrane phosphatide biosynthesis. In human, citicoline has more than 90% bioavailability, and is rapidly absorbed. Less than 1% is excreted in faeces (2).

“Produced endogenously, citicoline serves as a choline donor in the metabolic pathways for biosynthesis of acetylcholine and neuronal membrane phospholipids, mainly Phosphatidyl choline. As an exogenous compound, citicoline is also a source for acetylcholine synthesis, a key neurotransmitter and a member of the group of molecules that play important roles in cellular metabolism known as nucleotides “(1).

It is demonstrated that when a citicoline is taken orally or intravenously, it is completely absorbed into the blood circulation (3). Once absorbed, citicoline is widely distributed throughout the body and passes from the blood–brain barrier and reaches the central nervous system as a main target, where it is incorporated into phospholipids (4). Subsequently, citicoline enhances the biosynthesis of structural phospholipids that form neuronal
membranes, increases brain metabolism, and influences levels of multiple neurotransmitters (5). Stabilizing cell membrane, it can reduce ischemic injury by reducing free radical generation (6). Besides citicoline has antiedematous effects and decreases the formation of free radicals through suppressing the phospholipases (A1, A2, C–D) which provides the systems with antioxidant protection (7). Citicoline also has been proved to increase acetylcholine, norepinephrine and dopamine levels in the central nervous system (8), denoting it may have beneficial effects in conditions like head trauma of varying severity, cognitive disorders, Parkinson’s disease, glaucoma and strabismus (9). New studies have confirmed the role of citicoline in reversing different pathological conditions in canine medicine. Citicoline has only recently gained significant attention as a neuroprotective agent in dogs and it has been found to improve learning and memory (6). In a preliminary study, it has shown that use of citicoline prior to thiopental sodium anesthesia can improve brain function by decreasing the duration of lack of response to corneal reflex and also increasing the duration of analgesia (10). Appropriate dosage selection is much dependent on the pharmacokinetics of citicoline. This study was the first attempt to determine the blood levels of citicoline after IV and IM administration in different times in dogs.

Material and Methods

The subjects of this study were 12 male, mixed breed dogs that were approximately 2 to 3 years old and had a body weight between 15 and 25 kg. The animals were randomly divided into two treatment groups; intravenous and intramuscular. Dogs were considered healthy based on physical examination, complete blood count, serum biochemistry, and urine specific gravity before initiation of the study. Each dog received core vaccinations and routine deworming. All dogs were kept in an approved animal care facility for 2 weeks before and during the trial. The study was approved by and ran according to the faculty of veterinary medicine of Shiraz university guidelines.

Experimental chemicals and drugs:

The standard of citicoline (Sigma), HPLC grade water (Merck), acetonitrile (E. Merck Germany), citicoline injection (250mg/2ml ampules, AlborzDaru®) were used in this study.

Experiment:

Six dogs received an intravenous injection of 50 mg.kg-1 of citicoline via cephalic vein. Six additional dogs with the same weight range received an Intramuscular injection of 50 mg.kg-1 of citicoline in the quadriceps muscle. Blood samples (5 mL) were collected from all dogs in heparinized test tubes before and at 5, 15, 30, 60, 120, 180, 300 minutes and 24 hours after i.v and i.m administration. Plasma was harvested by centrifugation and stored frozen in sterile plastic vials until analysis.

HPLC:

The Knauer HPLC system consisted of Smartline pump (1000), equipped with a UV detector 2500 knauer set at 260 nm. The separation was carried out isocratically using a Knauer reverse phase C18 column model (vertex plus 250*4.6 mm Eurospher 100-5) with guard cartridge, column oven model CTO-6A knauer. The system was used at 20°C. Samples were eluted from the column at flow rate of 0.7ml min⁻¹. Solvents were degassed under vacuum. A
mobile phase of HPLC grade acetonitrile: water (20:80, v/v) was adjusted at pH 3.0 using 1% orthophosphoric acid. Both sample and standard solutions were filtered through 0.22 µm membrane filter. A sample volume of 20 microliter was injected to HPLC. Six standard concentrations were injected to obtain the standard curve. The resultant concentrations of 0.125, 1.25, 12.5, 62.5, 125 and 625 microgram/ml were used for drawing the standard curve ($R^2=0.9996$). Retention time was set at 3.0 ± 0.2 minutes (Figure 1). Plasma samples were extracted by protein precipitation with methanol. Serum (100µl) was mixed with 400µl of HPLC grade methanol, incubated for 3 minutes at 4˚C before centrifuging at 5000 rpm for 6 minutes. The clear phase was transferred to another polypropylene tube for injection to the HPLC device.

Results:

No clinical signs or drug adverse effect was noticed during and after the period of administration of citicoline.

Citicoline was detected in the plasma at different concentrations via I.V and I.M dosing in dogs. Table 1 summarizes the mean concentration of citicoline after I.V and I.M administration after different intervals.

<table>
<thead>
<tr>
<th>Time</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>300 min</th>
<th>1440 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>route</td>
<td>15.3 ±</td>
<td>18.7 ±</td>
<td>9.7 ±</td>
<td>12.9 ±</td>
<td>12.7 ±</td>
<td>14.6 ±</td>
<td>21.8 ±</td>
<td>10.7 ±</td>
</tr>
<tr>
<td></td>
<td>0.09</td>
<td>0.05</td>
<td>0.09</td>
<td>0.02</td>
<td>0.05</td>
<td>0.01</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>IM</td>
<td>6.1 ±</td>
<td>7.8 ±</td>
<td>7.1 ±</td>
<td>7.7 ±</td>
<td>11.6 ±</td>
<td>5.1 ±</td>
<td>11.6 ±</td>
<td>8.2 ±</td>
</tr>
<tr>
<td>route</td>
<td>0.04</td>
<td>0.09</td>
<td>0.07</td>
<td>0.06</td>
<td>0.06</td>
<td>0.04</td>
<td>0.06</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Fluctuation in the plasma concentration of citicoline sodium after both IV and IM administration showed that citicoline by both mentioned routes gave rise to two chromatographic peaks in plasma concentration. After intravenous administration, the first peak was at 15 minutes, and the second larger peak at 5 hours post-dosing (Figure 2). Furthermore, the peak concentration of citicoline were at 2 hours and 5 hours post dosing after intramuscular administration (Figure 3). These two peaks in concentration indicate that absorption into the systemic circulation is not instantaneous. Other related pharmacokinetic parameters weren’t measurable because of biphasic kinetics of this drug.
**Fig 1.** Retention time of the standard citicoline solution.

**Fig 2.** Plasma citicoline concentrations after different intervals post IV administration in dogs.
Fig 3. Plasma citicoline concentrations after different intervals post IM administration in dogs.

Figure 4 shows comparison of the citicoline concentrations in the blood of rats (11) and dogs (current study) after its IV administration from zero to 24 hours. Although the method used in pharmacokinetics of citicoline in rats differed from that of ours, a reasonable comparison can be made between the concentrations of the drug in both species at different times post IV administration. The two curves show somehow similar increase and decrease in concentration and also the peak levels in both species occur at the same time.

Fig 4. Comparison of the Plasma citicoline concentrations of dogs after IV administration with rats in the first 24 hours.

Discussion:
Estimation of the plasma concentrations of the drug via different administration routes at various intervals, let us to determine specific dosing regimens. In the present study, measuring plasma concentration of citicoline sodium after I.V and I.M administration showed biphasic plasma peaks which occurred at 15 minutes and 5 hours after I.V and at 2 and 5 hours after I.M administration.

Literature survey revealed that two methods such as HPLC (12-14) and Spectrophotometric (15-18) applied for the analysis of citicoline either as an individual drug in pure, pharmaceutical forms or in combination with impurities as well as in biological fluids. To achieve the highest precision in quantitative estimation of citicoline, here, a reversed phase liquid chromatography method was developed and validated. Method validation in terms of linearity, precision, accuracy, detection limit, quantification limit and robustness was performed. Our findings revealed that the kinetics pattern of citicoline was very similar to the human and rat after I.V administration. Pharmacokinetic studies using citicoline in humans showed elimination occurs mainly via respiratory and urinary excretion, in two phases mirroring the biphasic plasma peaks (1). The first peak in plasma concentration is followed by a sharp decline, which then slows over the next 4-10 hours. In the second phase, an initially rapid decline after the 24-hour plasma peak is similarly followed by a slower elimination rate. Similarly, in dogs the initial peak and subsequent decline was observed.

Present results showed that citicoline concentrations curves in different times after either IV or IM administration didn’t show conventional logarithmic manner, therefore we cannot discuss about half time and many other kinetics indexes. Plasma citicoline levels in this assessment showed a relative high concentration for 24 hours, which means slow elimination of citicoline from the body. It may favor prolonged therapeutic or possible toxic actions of the drug. Since no clinical signs or drug adverse effect was noticed during and after the period of administration of citicoline, we can assume it is a safe drug for canine medicine application. Drug safety and high plasma citicoline concentration persisted for a number of hours, so giving the drug once a day with 30-50 mg/kg dosage or twice daily with 15-30 mg/kg dosage would cause their levels to remain elevated for much of the day and doesn’t have any serious adverse effect. The current study is important for several reasons. Firstly, our data provides the first evidence that citicholine could administrate in dogs with the dosage regimen like human. Secondly, our data proposes a new method for assessment of citicholine kinetics in dogs.

Conclusion:

The proposed method of analysis provided a sensitive and specific assay for citicoline determination in dog plasma. The current data from citicoline kinetic in dogs and its safety showed the possibility to utilize the dosage used in humans, in canine patients according to the pharmacokinetics data.

References:


