Estimation of pharmacokinetic parameters of alpha-lipoic acid in the chicks model

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ABSTRACT

Background: Alpha-lipoic acid is a drug used to treat diabetic neuropathy, and it has other uses as a dietary supplement.

Objective: The target of the study was to investigate the concentration of therapeutic doses of Alpha-lipoic acid in the blood plasma of broiler chicks to define the pharmacokinetic parameters.

Methods: A randomized controlled study was performed on thirty-five healthy broiler chicks of seven days old, chicks were injected into the peritoneum with a single dose of analgesic ED$_{50}$ 80mg /kg b.wt, following injection of the drug, blood samples were collected at 0.25, 0.5, 1, 2, 4, 24 h (five chicks per time) from the jugular vein. Then the blood plasma was obtained, the concentrations of Alpha-lipoic acid in blood plasma samples were determined utilizing UV Spectrometric Method, the pharmacokinetic parameters were determined by the PKSolver program. Time versus concentration curve for Alpha-lipoic acid was obtained from the program. The pharmacokinetic parameters were determined with non-compartmental models.

Results: The concentration of Alpha-lipoic acid in the blood plasma of chicks injected with Alpha lipoic at a dose (80 mg/kg) were 134.6±7.17, 178.5±4.10,192.4±7.83, 158.5±11.05, 147.1±10.16, 122.8±7.09 µg/ml at times 0.25, 0.5, 1, 2, 4, and 24 hours respectively. The maximum plasma concentration was 192.4µg/ml during a period of 1 hour of injection. The terminal elimination half-life was 65hours, the terminal phase elimination rate constant was 0.011 h$^{-1}$, the mean residence time was 94h, and the area under the curve from time 0 to infinity was 14960 µg.h/ml.

Conclusions: Our study concluded that the peak of the analgesic effect of alpha lipoic acid was one hour after treatment, furthermore, it is characterized by a long elimination half-life and a poor clearance from the chick’s body, which is reflected in the long effects of its pharmacological properties

Keywords: Alpha-lipoic acid, Pharmacokinetic, Broiler chicks, UV spectrometric method
Introduction:
Chicks model have been involved as a research model extensively during the last decade; chicks have been utilized as an animal model for pharmacology and toxicity studies\textsuperscript{1,2}. The information about the pharmacokinetics of alpha-lipoic acid in the bird is poorly studied, it is important to study all aspects of drugs and preparations in most animal models in order to develop a clear picture of the properties of the drug used. Alpha-lipoic acid (ALA) is a natural product consisting of a 5-membered cyclic disulfide and a hydrocarbon tail that ends in a carboxylic acid group. Alpha-lipoic acid, as a result, is a predominantly hydrophobic particle with an amphipathic charisma because of the carboxylic acid group involved in the ring structure\textsuperscript{3}. Alpha-lipoic acid is found in our diet primarily in animal tissues like muscles and liver, and at low or untraceable levels in plant foods like potatoes. Alpha-lipoic acid, on the other hand, is considered desirable when used as a food additive due to its antioxidative activity, which has initially been described, and many articles have indicated its preventive properties in cases such as aging, type 2 diabetes, and neuropathy\textsuperscript{4}. Many studies have confirmed that Alpha-lipoic acid has analgesic effects for pain. Alpha-lipoic acid has an inhibitory effect on calcium channels type V3.2 in sensory neurons of the dorsal root ganglia of rats as well as reported that local injection of ALA reduced the sensation of heat and mechanical stimulation\textsuperscript{5}. Alpha-lipoic acid works to relieve visceral pain in rats\textsuperscript{6}. The analgesic mechanism in this research is due to ALA inhibiting sodium channels, blocking them, and reducing their cellular synthesis, especially sodium channels of type V1.8 located in the sensory nerves of the colon in rats with diabetes mellitus. To the best of our knowledge, there is no pharmacokinetic study of Alpha-lipoic acid in chick’s model. Therefore, the aim of our study is to define the pharmacokinetic parameters of alpha lipoic acid in this animal model.

Materials and methods:
Animals:
Ross broiler chicks of unsexes were obtained at age of one day from a local hatchery in Nineveh, Iraq. They were housed in batches of 20-25 chicks at a temperature of 25-30 °C with 24 h lighting and wood shavings as floor litter. The supply of water and feed were ad libitum. Experiments were conducted when the ages of the chicks were at the 8 days.

Ethical approval:
The research, which is part of a master’s thesis, was approved by the Scientific Committee of the department of the Physiology, Biochemistry and Pharmacology of the College of Veterinary Medicine at the University of Mosul. The ethical approval number UM.VET.2022.6.
Doses selection

The doses were chosen according to the determination of the median effective dose for analgesia ED$_{50}$. The ED$_{50}$ of the Alpha lipoic acids was calculated by the up and down method described by Dixon$^7$. Hence the authors selected one of Alpha-lipoic acid 80 mg/kg.

Calculation of the median Effective dose ED$_{50}$ of alpha-lipoic acid in chicks

Determination of the median effective dose (ED$_{50}$) corresponds to a degree of the influence of a drug, being the dose of a drug necessary to yield 50% of that drug’s maximal effect of Alpha-lipoic acid for the induction of analgesia in chicks by electrical and thermal stimulation. Chicks at 7-9 days old were used.

This method is summarized by injecting a chick with a dose of Alpha-lipoic acid and then examining the analgesic effect 15 minutes after the injection. If analgesia appears, the chick is given an X mark and if it does not happen, the chick is given the mark O, and by repeating this method up and down the dose by a fixed amount (20mg/kg) after the change in effect was occurring enabling us to calculate the median effective dose (ED$_{50}$) of Alpha-lipoic acid based on the table mentioned$^7$ and Using the following equation:

ED$_{50} = X_f + K_d$

Whereas

X$_f$ = the last dose used in the experiment.

K = a tabular value extracted from the table mentioned by Dixon$^7$.

d = the amount of constant increase or decrease in the administered dose.

Hot water test

This test is based on the principle of thermal stimulation, where we used a water bath device and its temperature was set at 55-56 C° by the internal thermostat of the device. The chick was grabbed gently in one hand, and his left foot was placed under the fingers, and the right foot was left free to move and dipped to before the tarsal joint. The time spent per second was measured by a stopwatch, if the chick does not respond to the thermal stimulus within 20 seconds, it is immediately raised and its right foot is placed in water at room temperature for 15 seconds to quickly reduce the leg temperature and was then saw for possible heat-induced burns$^8$.

Measuring the concentration of alpha-lipoic acid in blood plasma by means of a spectrophotometer
This experiment was conducted using 30 chicks, 8 days old, and their weights ranged between 85-110 g. A dose of 80 mg/kg of body weight was used, which is represent analgesic ED\textsubscript{50}. All chicks were injected with this dose and blood samples were collected to obtain Blood plasma at times 0.25, 0.5, 1, 2 and 24 hours, with 5 chicks for each time, to measure the concentration of Alpha-lipoic acid during the mentioned times (Patni and Rawat, 2018).

**Blood plasma samples extraction**

1. All chicks were injected with Alpha-lipoic acid in a single dose of 80 mg/kg Ip.
2. Then blood samples were taken through the jugular vein from the estimated time for every five chicks 0.25, 0.5, 1, 2, 4 and 24 hours after the injection.
3. After that, blood plasma was obtained by adding heparin to blood samples in a ratio of (10:1) by diluting it with a physiological solution.
4. The samples were placed in a centrifuge at a speed of 3000 rpm for 15 minutes, then the blood plasma was separated and the plasma samples were then frozen at -18°C for 72 hours for the purpose of conducting the analysis after that by means of a spectrophotometer.

**Preparation of phosphate-buffered solution**

1. The phosphate-buffered solution was prepared by dissolving 6.82 g of monopotassium dihydrogen phosphate KH\textsubscript{2}PO\textsubscript{4} in 250 ml of distilled water in a graduated flask.
2. For preparation of 0.2 molarity of KH\textsubscript{2}PO\textsubscript{4}. Another graduated flask was used to prepare NaOH (0.2 M) by dissolving 2 g of NaOH in 250 ml of the distilled water.
3. Then 195.5 ml of NaOH solution was taken and added to the previously prepared solution of KH\textsubscript{2}PO\textsubscript{4}, then the volume of the solution was completed to 1000 ml by adding distilled water.
4. The pH function was checked by means of a pH meter to reach a buffer solution with a pH of 7.4 by treating the solution with an acidic substance HCl or a base substance NaOH as needed to reach a pH 7.4.

**Preparation of standard solutions**

1. Standard solutions of alpha lipoic acid were prepared at concentrations of 50, 100, 200, 400 and 800 µg/ml and diluted with the prepared buffer phosphate solution.
2. To obtain 5 ml for each concentration, then the solution was filtered by means of a filter paper, and then the pure solution was analyzed by a spectrophotometer at a wavelength of 330 nm (source) to extract the equation of the simple regression line attached.

**Extraction of alpha-lipoic acid from blood plasma samples**
Alpha lipoic acid was extracted from blood plasma samples using an accurate and approved method.

1. We added 1 ml of the previously prepared phosphate buffer solution to 1 ml of the blood plasma sample and mix them in a glass tube.

2. Then we putted the tube in the centrifuge at 3500 rpm for 10 minutes.

3. Then the tube was extracted and the solution was filtered by a qualitative filter paper.

4. After that, the solution was taken and measured by means of a spectrophotometer at a wavelength of 330 nm, and the device was calibrated by means of a buffer phosphate solution (Singh et al., 2016).

Measuring the pharmacokinetics of alpha-lipoic acid in chick blood plasma

The pharmacokinetics were calculated and measured after identifying the concentration of alpha-lipoic acid in the blood plasma from the previous experiment and during different times using a spectrophotometer.

The pharmacokinetic parameters of alpha-lipoic acid were calculated by using the PKSolver program (Exel)(Patni and Rawat, 2018).

While the pharmacokinetic criteria were calculated and compared with the results of the program using the following equations:

1- Elimination rate constant (kel): It is the fixed percentage of the drug that is removed from the blood plasma per hour.

2- Elimination Half-Life (t1/2β): It represents the hourly time required for the drug concentration in the blood plasma to decrease by 50%.

Subtraction half-life (hour) = 0.693 / subtraction rate constant

3- Volume of distribution (Vd): The volume of apparent body fluids that serves to contain the drug.

Diffusion volume (L/kg) = dose (mg) / drug concentration at time 0 (C0)

4- Total Clearance (Cl): It is represented by the efficiency of the various parts of the body in filtering and excreting the drug.

Total filtration (liters/hour/kg) = volume of diffusion x constant rate of subtraction

5- Area under plasma concentration-time Curve (AUC): The drug concentration in the blood plasma over different time periods.

Area under the time-concentration curve for blood plasma (µg x h/ml) = dose (mg)/total clearance
6- Area under Moment Curve (AUMC): It is the concentration of the drug in the blood plasma at the moment of measurement.

Area under the instantaneous curve (µg x h2/ml) = volume of diffusion x (area under the time-concentration curve for blood plasma) / dose (mg)

7- Mean Residence Time (MRT): It is the expected period for the survival of alpha-lipoic acid in the blood plasma.

Average residence time (hours) = area under the instantaneous curve / area under the time-concentration curve for blood plasma

8- Time Maximum (Tmax): It is the time during which the drug concentration in the blood plasma reaches its highest level.

9- Concentration Maximum (Cmax) (µg/ml): It represents the highest concentration of the drug in the blood plasma during a certain time.

Results:

Determination of the median effective dose ED₅₀ for analgesia of Alpha-lipoic acid in chicks:

The median effective analgesic (ED₅₀) values of Alpha-lipoic acid in the chicks by hot water test was 77.6 mg/kg IP (Table 1).

Table 1: Median effective dose (ED₅₀) of ALA injected IP for generation of analgesia in chicks by using thermal stimulation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED₅₀ mg/kg</td>
<td>77.6</td>
</tr>
<tr>
<td>Average of the doses used mg/kg</td>
<td>100-60=40</td>
</tr>
<tr>
<td>Initial dose mg/kg</td>
<td>100</td>
</tr>
<tr>
<td>Last dose mg/kg</td>
<td>60</td>
</tr>
<tr>
<td>Increase or decrease in the dose mg/kg</td>
<td>20</td>
</tr>
<tr>
<td>Number of chicks involved</td>
<td>(XOXXO) 5</td>
</tr>
</tbody>
</table>
Dose sequence | 100-80-100-80-60
---|---
Equation application: $ED_{50} = X_f + K_d$ | $60 + (0.878 \times 20) = 77.6$
Heat temperature of water bath | 55-56 °C
Sings of nociception | Foot withdrawal

We calculated the time needed to foot withdrawal from water bath before and after 15 min of Alpha-lipoic acid injection

X: Positive reaction of analgesia, O: Negative reaction of analgesia, The $ED_{50}$ were calculated by the up-and-down method

**Measuring the concentration of Alpha-lipoic acid in blood plasma by a spectrophotometer**

The administration of alpha-lipoic acid at 80 mg/kg of body weight intraperitoneally led to its appearance in the blood plasma of chicks with concentrations: 134.5, 178.6, 192.4, 158.5, 147.1 and 122.8 micrograms/ml at times 0.25, 0.5, 1, 2, 4 and 24 hours, respectively, and the concentration of alpha-lipoic acid was high at times half an hour after injection and then decreased to reach its lowest concentration after 24 hours of administration (Figure 1).

**Measuring the pharmacokinetics of Alpha-lipoic acid in chick blood plasma**

The pharmacokinetic parameters of alpha-lipoic acid in the blood plasma of injected chicks at a dose of 80 mg/kg are represented by the area under the curve was 14960.7 μg/ml*h and Area under the instantaneous curve was 1417579.6 μg h2/ml, and the mean residence time was 94.8 hours. Elimination rate constant was 0.0106 h-1, the volume of distribution was 0.507 L/kg, the total clearance was 0.0054 L/hr/kg, the half-life was 65.7, the time maximum was 1 hour, and the maximum concentration was 192.4 µg/ml (Table 2).
Table 2: Pharmacokinetic parameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>kel</td>
<td>1/h</td>
<td>0.010557143</td>
</tr>
<tr>
<td>t1/2</td>
<td>h</td>
<td>65.65670168</td>
</tr>
<tr>
<td>Tmax</td>
<td>h</td>
<td>1</td>
</tr>
<tr>
<td>Cmax</td>
<td>μg/ml</td>
<td>192.4</td>
</tr>
<tr>
<td>AUC</td>
<td>μg/ml*h</td>
<td>14960.67252</td>
</tr>
<tr>
<td>AUMC</td>
<td>μg/ml*h^2</td>
<td>1417579.626</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>94.75373675</td>
</tr>
<tr>
<td>Vd</td>
<td>(mg)/(μg/ml)</td>
<td>0.506515186</td>
</tr>
<tr>
<td>Cl</td>
<td>(mg)/(μg/ml)/h</td>
<td>0.005347353</td>
</tr>
</tbody>
</table>
Discussion:

The alpha-lipoic acid is a reliable antioxidant agent used in oxidative stress-related clinical conditions like (diabetes mellitus, neuropathy, neurodegenerative disease, and cardiovascular disorder, obesity vertigo, and others). Our study determines the pharmacokinetic parameters of the intraperitoneal dose alpha-lipoic acid at 80mg/kg bw in broiler chicks. Our findings are in agreement with studies carried out on rats and humans.

When ALA is given orally or intravenously, it is metabolized in the body after absorption from the stomach and small intestine and spreads to the liver through the portal circulation and to the rest of the body through the systemic circulation. ALA is soluble in water, hydrophilic and lipophilic, so it can cross the blood-brain barrier and can exist both inside and outside the cell and inside mitochondria. The half-life in plasma is about 30 minutes after oral dosing in dogs, and the LD50 was about 400-500 mg/kg body weight. Gastrointestinal absorption is variable and decreases with food so it is recommended that ALA be taken 30-60 minutes before or at least 120 minutes after the meal. ALA reaches its maximum levels in the blood plasma 30-60 minutes after ingestion and is believed to be metabolized in the liver. It is recommended to take ALA on an empty stomach in order to take advantage of the acidic environment of the stomach, which is necessary to enhance gastric absorption of a weak acid such as ALA and to reduce competition with nutrients. Others during intestinal absorption. Despite this and the progress that has been
made with different solid pharmaceutical formulations (tablets, coated granules, delayed-release, and rapid release)\(^{22,23}\), increasing plasma concentration and stabilizing ALA remains an objective. It must be achieved. Studies accumulated over the past years have confirmed the fact that ALA is poorly soluble in an aqueous and acidic environment such as that of the stomach. This affects the concentration available for absorption, and thus represents an important concomitant cause of low bioavailability after oral administration. Moreover, the first section of the intestine is involved in the absorption of the remainder of ALA through specific carrier proteins \(^{20}\). ALA is absorbed in low concentrations by active transport and mediated by carriers, and certainly in competition with short-chain fatty acids, but if it is present in high concentrations, it is absorbed by diffusion\(^{24}\).

A pharmacokinetic study conducted in humans showed that the salt-bound form of ALA, which is sodium ALA, has a pharmacokinetic difference from that of the free form of ALA\(^{25}\).

A study conducted on rats to measure the kinetic parameters of ALA using subcutaneous injections at doses of 20, 50, and 100 mg/kg body weight revealed the following kinetic parameters: the maximum plasma C\(_{\text{max}}\) and AUC were 3.8 and 443.1 \(\mu\)g/ml for the group The first and 9.9 and 745.2 \(\mu\)g/ml for the second group and 10.3 and 848.8 \(\mu\)g/ml for the third group, respectively\(^{26}\), and another study conducted on rats indicated that the pharmacokinetics of the racemic mixture of ALA are R\(_{\text{ALA}}\) and S\(_{\text{ALA}}\) when given them This study showed that the concentration of R\(_{\text{ALA}}\) in the plasma was higher than the concentration of S\(_{\text{ALA}}\) in the plasma, and the area under the curve for R\(_{\text{ALA}}\) was 1.26 greater than S\(_{\text{ALA}}\), and when they were given together in the same proportion by intravenous administration, there was no significant change between the kinetic parameters of both R\(_{\text{ALA}}\) and S\(_{\text{ALA}}\) \(^{17}\). The practical application of the pharmacokinetics criteria that we obtained may be of clinical benefit to determine the therapeutic doses and not to reach the toxic doses. To our knowledge, this study is the first study of the pharmacokinetics of alpha-lipoic acid in an avian model.

**Conclusion:**

Our finding concluded that the intraperitoneal administration of alpha-lipoic acid to chicks has an analgesic effect, and the pharmacokinetics parameters for it after one hour are characterized by the relatively long duration of its effect, long elimination half-life and a poor clearance from the chick’s body tissues.

**Competing of interest**

The authors declare that they have no competing interests.

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