JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2019.f371
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

December 2019
VOL 6, NO. 4, PAGES 481–485

SHORT COMMUNICATION

Pharmacokinetics of intravenous administered two different high doses of ascorbic acid in healthy beagle dogs

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ABSTRACT

Objective: We performed a randomized two-way crossover study to evaluate the pharmacokinetic profiles of two high-dose ascorbic acid (AA) after IV infusion in healthy beagle dogs.
Materials and Methods: The dogs were administered IV AA at two doses of 1.5 and 3 gm/kg for 4 h, and the AA concentration in plasma and urine pH was measured before and after administration.
Results: The plasma concentrations of AA in both groups peaked 3 h after administration. Among the two groups, the urine pH was not significantly different (p = 0.1299–0.7944). High-dose IV AA did not induce serious adverse events in dogs.
Conclusion: The results of this study suggest that the high dose of AA which reaches the therapeutic dose for cancer and supports the safety of high-dose IV AA in dogs.

ARTICLE HISTORY

Received August 18, 2019
Revised September 05, 2019
Accepted September 08, 2019
Published October 23, 2019

KEYWORDS

Ascorbic acid; dog; pharmacokinetics.

Introduction

Ascorbic acid (AA) is used at a high dose to selectively kill cancer cells when the plasma concentration exceeds 0.3–20 mM [1–3]. AA as a co-factor for hydroxylases is required to impede the activity of the transcription factor hypoxia-inducible factor-1 (HIF-1), which affects cell death and survival pathways [4,5]. High doses of IV AA increase plasma and tumor AA concentrations, which are maintained for three times longer than that in plasma. Increased tumor AA concentration decreases, and vascular endothelial growth factor levels and HIF-1, thus, exhibiting anticancer effects [6]. Oral administration of high-dose AA does not result in drug therapeutic levels since the absorption of AA in the intestine is tightly controlled [7], but IV infusion of AA can achieve therapeutic plasma concentration [8]. Therefore, high-dose IV AA is used as an alternative treatment for cancer [9]. It has been reported to reduce the rate of tumor growth in both mice and humans [6,10,11]. In the pharmacokinetic study of high-dose IV AA, veterinary literatures are very limited. The objective of this study was to evaluate the pharmacokinetic profiles and safety of high-dose AA after IV infusion and determine the potential therapeutic concentration of AA in healthy dogs, so that high-dose AA may be applied to treat cancer in dogs.

Materials and Methods

Ethical approval

The current study was approved by Institutional Animal Care and Use Committee at Chungnam National University (approval number CNU-16-1047).

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How to cite: Lim S, Oh Y-I, Jeong J-W, Song K-H, Koo T-S, Seo K-W. Comparison of the pharmacokinetics of two high doses of intravenous ascorbic acid in healthy beagle dogs. J Adv Vet Anim Res 2019; 6(4):481–485.

Animals and sample collection

Dogs were randomly assigned to two treatment groups in each of the two periods of the study, using a randomized two-way crossover design. AA was administered to each dog with a washout period of at least 7 days between experiments. Food was withheld for 12 h before infusions. Water was provided freely before, during, and after the infusion. Group A and B dogs received a single constant rate infusion (CRI) of 1.5 and 3 gm/kg AA, respectively, for 4 h. For the infusion, AA was diluted in sterile water to achieve an osmolarity between 700 and 1,000 mOsm/l. The infusate was placed in an opaque bag, and the infusion rate was maintained below 1 $g \cdot kg^{-1} \cdot h^{-1}$. Blood samples were collected immediately before infusion, during infusion (1, 1.5, 2, and 3 h), and after the endpoint (4 h, 4 h 20 min, 4 h 40 min, 5 h, 6 h, 8 h, 10 h, and 14 h). Urine samples were collected before infusion and 10 h, 24 h, and 7 days after infusion via cystocentesis or urinary catheter. Urine pH was determined using a urine stick just after the collection. Urine sediment was microscopically examined to confirm the presence of oxalate crystals.

Measurement of plasma AA concentration

Plasma concentration of L-ascorbic acid (L-AA) was detected by using liguid chromatography-tandem mass spectrometry (LC-MS/MS). For the sample preparation, 2 µl of plasma was processed with 176 µl of acetonitrile and 20 µl of internal standard solution (1 µg/ml of 5-fluorouracil in methanol) to induce protein precipitation. The sample mixture was then centrifuged for 10 min at 17,600×g right after vigorous vortex mix for 10 min. The supernatant was transferred to a sample vial and 5 µl injected into the LC-MS/MS analysis system. Injected samples were chromatographically separated on a C_{18} column (DIKMA SpursilTM C_{18} 3.5 µm, 2.1 × 100 mm) by an Agilent 1100 high-performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA). Separated L-AA and 5-fluorouracil were analyzed by a triple quadrupole mass spectrometry (4000 QTRAP, Applied Biosystems/MDS SCIEX, Foster City, CA), the negatively charged ions were detected by multiple reaction monitoring mode under the transitions of m/z 174.98 to 115.00 for L-AA and m/z 128.88 to 42.10 for 5-fluorouracil. The peak areas for L-AA and 5-fluorouracil were calculated using Analyst® 1.6.2 Software (Applied Biosystems/MDS SCIEX). L-AA and 5-fluorouracil were detected at 0.76 and 0.79 min, respectively. To quantify L-AA, calibration curves were established based on the peak area ratios (L-AA/5-fluorouracil) versus the nominal concentration of the calibration standards by using a least-squares quadratic regression analysis with weighting factor ($1/x^2$).

Analysis of data and statistics

To estimate the pharmacokinetic parameters for L-AA, the plasma concentration versus the time profiles were analyzed using non-compartmental model analysis with Phoenix WinNonlin software version 6.2 (Pharsight, Cary, NC) program. The elimination rate constant (k_e) was estimated using linear regression for the log-linear portion of the terminal phase. The terminal elimination half-life ($T_{1/2}$) was obtained by dividing 0.693 by k_e. The area under the L-AA plasma concentration versus the time curve from time zero to infinity ($AUC_{0-\infty}$) and the area under the respective first moment time curve from time zero to infinity ($AUMC_{0-\infty}$) were calculated using the linear trapezoidal rule and the standard area extrapolation method using WinNonlin 6.2.

The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly computed from the plasma concentration-time curves. The test for normality was performed using the Kolmogorov–Smirnov test. Mann–Whitney *U* test as a nonparametric method was used to compare pharmacokinetic parameters between the two groups using Prism 6 Version 6.01 (GraphPad). Statistical significance was determined as $p < 0.05$.

To estimate the pharmacokinetic parameters for L-AA, the plasma concentration versus the time profiles were analyzed using non-compartmental model analysis with Phoenix WinNonlin 6.2 Software (Pharsight, Cary, NC) program. The elimination rate constant (k_e) was estimated using linear regression for the elimination phase on a semi-log scale. The elimination half-life ($T_{1/2}$) was obtained by the division of 0.693 by k_e. The area under the L-AA plasma concentration versus the time curve from time zero to infinity ($AUC_{0-\infty}$) and the area under the respective first moment time curve from time zero to infinity ($AUMC_{0-\infty}$) were calculated using the linear trapezoidal method and the standard area extrapolation method using WinNonlin 6.2. The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly computed from the temporal plasma concentration curves. The test for normality was performed using the Kolmogorov–Smirnov test. Mann–Whitney *U* test as a nonparametric method was used to compare pharmacokinetic parameters between the two groups using Prism 6 Version 6.01 (GraphPad). When the *p* value was less than 0.05, the results were considered as statistically significant.

Results

Figure 1 illustrates the mean plasma concentration of AA during and after infusion, which increased and peaked at 3 h during AA CRI. From 1 to 8 h after the administration of AA, the plasma concentrations in both groups continuously remained above the minimum pharmacologic

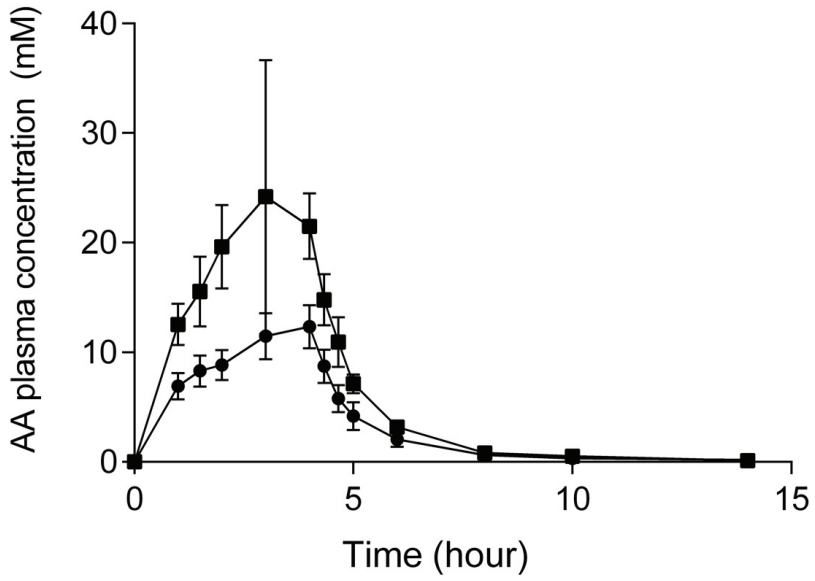

Figure 1. Plasma concentration (mean ± SD) after intravenous administration of high-dose ascorbic acid (AA) for 4 h to six healthy beagle dogs.

Table 1. Pharmacokinetic parameters of two high-dose ascorbic acid IV infusion in six healthy beagle dogs (mean ± SD).

Pharmacokinetic parameters	1.5 gm/kg	3 gm/kg	*p* value
C_{max} (mM)	12.44 ± 0.18	26.86 ± 9.33	0.002
T_{max} (h)	3.58 ± 1.02	3.50 ± 0.55	0.546
$T_{1/2}$ (h)	2.42 ± 1.08	1.74 ± 0.65	0.132
AUC (mM·h)	48.98 ± 7.61	93.49 ± 18.00	0.002

C_{max} = maximum plasma concentration, T_{max}, = time to reach C_{max}, $T_{1/2}$ = half-life, AUC = area under plasma concentration and time curve. *p*-value indicates comparison between two groups (1.5 and 3 gm/kg).

concentration. Plasma concentrations of AA after the 1.5 and 3 gm/kg doses were significantly different from 1 to 6 h after the administration (p = 0.0022–0.0043) but not at 8, 10, and 14 h. The pharmacokinetic parameters of high-dose IV AA infusion are shown in Table 1. There were significant differences in C_{max} and AUC between the two groups. Mean urine pH values at 0 h, 10 h, 24 h, and 7 days after AA infusion are presented in Figure 2. The difference in urine pH according to time was also not significant between the groups (p = 0.1299–0.7944). In the urine sediment test, crystals including oxalate were not observed. Mild diarrhea as an adverse event was observed in only one dog receiving AA at 3 gm/kg.

Discussion

To the best of authors' knowledge, this is the first investigation of the pharmacokinetic profile of high-dose IV AA in the plasma of healthy dogs, even though we conducted *in vitro* and confirmed the antitumor activity of high-dose AA [12]. In this study, both groups maintained plasma AA levels above the minimum therapeutic concentration (0.3 mM) from 1 to 10 h after administration and no adverse events were noted. The time for which the plasma concentration remained above 10 mM was 2 and 4 h 30 min at 1.5 and 3 gm/kg, respectively. Therefore, 3 gm/kg may be more suitable than 1.5 gm/kg to achieve approximate human pharmacologic concentration in dogs.

There was a significant difference in plasma concentration between the 1.5 and 3 gm/kg groups for up to 6 h after AA administration in this study. This is consistent with the findings of a previous human clinical study [13]. Therefore, when administered IV, the dose is considered to be a factor affecting the plasma concentration of AA. In our study, only one method of CRI for 4 h was used, but various doses, durations, and intervals of AA administration are required.

We compared the changes in urine pH before and after AA administration. It is suggested that the calcium oxalate stone formation occurs because of the excretion of oxalate into the urine acidified by AA [14]. The urine pH decreased after administration in four dogs in this study. However, it was not significant in the change of urine pH and no oxalate was found in the urine after AA administration. In this study, we investigated the effect of only single doses on urine pH and oxalate crystal formation through short-term CRI. The production of urine oxalate not only depends on urine AA (18%) but also on other metabolic factors, including thiamine [13]. Therefore, it is difficult to assess the impact of high-dose AA on urine. It might be necessary to monitor serial urine pH and oxalate crystal formation in patients receiving periodic AA therapy.

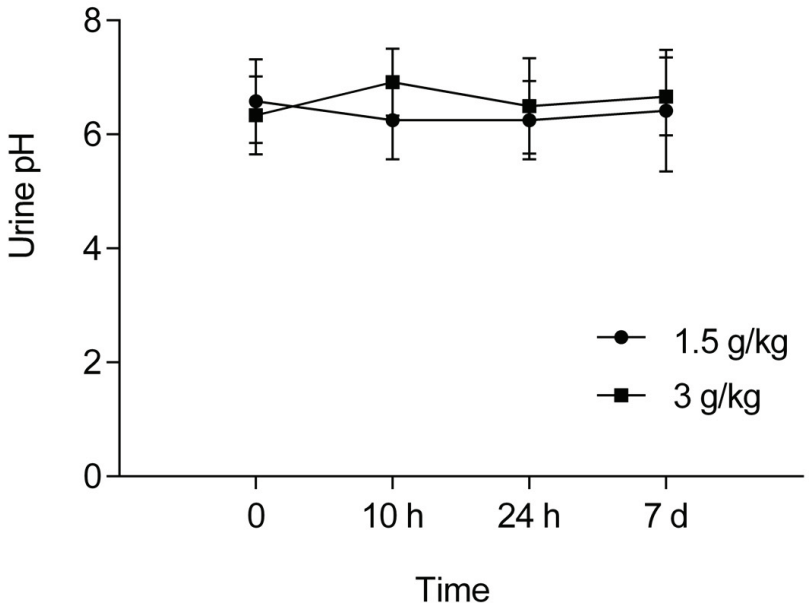

Figure 2. Alteration of urine pH after intravenous administration of 1.5 and 3.0 gm/kg ascorbic acid (AA, mean ± SD) to six healthy beagle dogs.

This study has some limitations that are worth mentioning. First, the pharmacokinetic analysis was performed only in healthy beagle dogs. A large-scale study is needed to evaluate the pharmacokinetic profile of high-dose IV AA in dogs with cancer. AA clearance from tumor tissues is slower than that from the plasma, and AA pharmacokinetics in cancer patients may differ from that in healthy dogs. Second, we could not investigate the effects of high-dose IV AA on organs in dogs. Adverse events of high-dose IV AA have not been studied in dogs to date. The study of the long-term effects of high-dose AA on the organs needs to establish criteria of indication.

Conclusion

In conclusion, high-dose (1.5 and 3 gm/kg) IV AA infusion achieved pharmacologic concentration in healthy dogs. AA was well tolerated in all the treated dogs without any severe adverse events. These results support that high-dose IV AA infusion could be beneficial in dogs with cancer.

Acknowledgements

This manuscript is written based on the Master's thesis of author Seula Lim. This research was carried out with the support of "cooperative Research Program of Center for Companion Animal Research (Project No. PJ1404522018): Rural Development Administration, Republic of Korea.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contribution

SAL performed the experimental procedures and drafted the manuscript. YIO interpreted the data and performed statistical analysis and revised the manuscript critically. JWJ performed the pharmacokinetic and the statistical analysis. KHS participated in scientific discussions. TSK contributed to design the study and revised the manuscript. KWS designed the experiment and overall coordinated the study.

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