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.

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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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Dogs were randomly assigned to two treatment groups in each of the two periods of the study, using a random-ized 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 g/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·kg⁻¹·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, 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 liquid chromatography-tan-dem 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 C₁₈ column (DIKMA SpursilTM C₁₈ 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 using a least-squares quadratic regression analysis with weight-ing factor (1/x²).

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 (kₑ) was estimated using linear regression for the log-linear portion of the terminal phase. The terminal elimination half-life (Tₑ½) was obtained by dividing 0.693 by kₑ. The area under the L-AA plasma concentration versus the time curve from time zero to infinity (AUC₀-∞₀) and the area under the respective first moment time curve from time zero to infinity (AUMC₀-∞₀) were calculated using the linear trapezoidal rule and the standard area extrapolation method using WinNonlin 6.2.

The maximum plasma concentration (Cₘₐₓ) and time to reach Cₘₐₓ (Tₘₐₓ) were directly computed from the plasma concentration-time curves. The test for normal-ity 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 (kₑ) was estimated using linear regression for the elimination phase on a semi-log scale. The elimination half-life (Tₑ½) was obtained by the division of 0.693 by kₑ. The area under the L-AA plasma concentration versus the time curve from time zero to infinity (AUC₀-∞₀) and the area under the respective first moment time curve from time zero to infinity (AUMC₀-∞₀) were calculated using the linear trapezoidal method and the standard area extrapolation method using WinNonlin 6.2. The maximum plasma concentration (Cₘₐₓ) and time to reach Cₘₐₓ (Tₘₐₓ) were directly computed from the temporal plasma concentration curves. The test for normal-ity 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
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 $C_{\text{max}}$ 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.

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**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 |
|-----------------------------|-----------|---------|-----------|
| $C_{\text{max}}$ (mM)       | 12.44 ± 0.18 | 26.86 ± 9.33 | 0.002     |
| $T_{\text{max}}$ (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     |

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

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**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.

**References**

[1] Chen Q, Espey MG, Krishna MC, Mitchell JB, Corpe CR, Buettner GR, et al. Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. Proc Natl Acad Sci USA 2005; 102(38):13604–9; https://doi.org/10.1073/pnas.0506390102

[2] Chen Q, Espey MG, Sun AY, Lee J-H, Krishna MC, Shacter E, et al. Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo. Proc Natl Acad Sci USA 2007; 104(21):8749–54; https://doi.org/10.1073/pnas.0702854104

[3] Verrax J, Calderon PB. Pharmacologic concentrations of ascorbate are achieved by parenteral administration and exhibit antitumor effects. Free Radic Biol Med 2009; 47(1):32–40; https://doi.org/10.1016/j.freeradbiomed.2009.02.016

[4] Kuiper C, Dachs GU, Carrie MJ, Vissers MC. Intracellular ascorbate enhances hypoxia-inducible factor (HIF)-hydroxylase activity and preferentially suppresses the HIF-1 transcriptional response. Free Radic Biol Med 2014; 69:308–17; https://doi.org/10.1016/j.freeradbiomed.2014.01.033

**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.
[5] Kuiper C, Vissers M. Ascorbate as a co-factor for Fe-and 2-oxoglutarate dependent dioxygenases: physiological activity in tumor growth and progression. Front Oncol 2014; 4:359; https://doi.org/10.3389/oncology.2014.00359

[6] Campbell EJ, Vissers MC, Wohlrab C, Hicks KO, Strother RM, Bozonet SM, et al. Pharmacokinetic and anti-cancer properties of high dose ascorbate in solid tumours of ascorbate-dependent mice. Free Radic Biol Med 2016; 99:451–62; https://doi.org/10.1016/j.freeradbiomed.2016.08.027

[7] Graumlich JF, Ludden TM, Conry-Cantilena C, Cantilena LR, Wang Y, Levine M. Pharmacokinetic model of ascorbic acid in healthy male volunteers during depletion and repletion. Pharm Res 1997; 14(9):1133–9.

[8] Nielsen TK, Højgaard M, Andersen JT, Poulsen HE, Lykkefeldt J, Mikines KJ. Elimination of ascorbic acid after high-dose infusion in prostate cancer patients: a pharmacokinetic evaluation. Basic Clin Pharmacol Toxicol 2015; 116(4):343–8; https://doi.org/10.1111/bcpt.12323

[9] Lee W-J. The prospects of vitamin C in cancer therapy. Immune Net 2009; 9(5):147–52; https://doi.org/10.4110/in.2009.9.5.147

[10] Chen Q, Espey MG, Sun AV, Pooput C, Kirk KL, Krishna MC, et al. Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice. Proc Natl Acad Sci USA 2008; 105(32):11105–9; https://doi.org/10.1073/pnas.0804226105

[11] Hoffer L, Levine M, Assouline S, Melnychuk D, Payatty S, Rosadiuk K, et al. Phase I clinical trial of iv ascorbic acid in advanced malignancy. Ann Oncol 2008; 19(11):1969–74; https://doi.org/10.1093/annonc/mdn377

[12] Shin H, Nam A, Song KH, Lee K, Rehn RB, Seo KW. Anticancer effects of high-dose ascorbate on canine melanoma cell lines. Vet and Comp Oncol 2018; 16(4):616–21; https://doi.org/10.1111/vco.12429

[13] de Grooth HJ, Manubulu-Choo WP, Zandvliet AS, Spoelstra-de Man AM, Girbes AR, Swart EL, Oudemans-van Straaten HM. Vitamin C pharmacokinetics in patients who are critically ill: a randomized trial of four iv regimens. Chest 2018;153:1368–77; https://doi.org/10.1111/vco.12429

[14] Bartges J, Kirk C. Nutritional management of lower urinary tract disease. Applied veterinary clinical nutrition. John Wiley & Sons, Ames, IA, pp 269–83, 2012.