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Serum concentration and safety of intravenous drip versus subcutaneous administration of carboplatin in dogs

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ABSTRACT. Carboplatin is used to treat certain cancers in dogs and cats and is routinely administered via intravenous drip (IVD). Subcutaneous (SC) administration has also been described. However, the toxicity, serum concentrations, and area under blood concentration-time curves (AUCs) of SC carboplatin are unknown. This study aimed to compare serum carboplatin concentrations in dogs after SC and IVD and to monitor any adverse events. In this crossover study, five dogs received SC or IV carboplatin (300 mg/m^2). After a minimum of 3 weeks, each dog received the other treatment. No gross skin toxicity or abnormal clinical signs were observed in any of the dogs. Blood test abnormalities were detected in most dogs. Decreased neutrophil and platelet counts, and increased C-reactive protein (CRP) levels were found. There was no significant difference in the neutropenia, thrombocytopenia, and CRP scores between the groups. Systemic toxicities of SC carboplatin were comparable to those of IVD carboplatin. The time to maximum carboplatin concentration after SC was longer than that after IVD (P<0.001). SC carboplatin remained in the serum longer than IVD carboplatin (P=0.008). The AUC of SC was less than that of IVD (P=0.002). The AUC and time taken to reach the maximum concentration of SC carboplatin were lower than those of IVD carboplatin. This study suggests that SC carboplatin may be an efficacious option for the treatment of tumors in dogs, particularly where IVD administration is challenging.

KEY WORDS: carboplatin, dog, route of injection, subcutaneous administration, veterinary medicine

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Carboplatin, a second-generation platinum chemotherapy drug, is used to treat certain cancers in dogs and cats, such as canine osteosarcomas, melanomas, carcinomas, and sarcomas [3]. Most chemotherapeutic agents can cause varying degrees of local tissue injury when extravasated [7]. Carboplatin is administered intravenously and is classified as an extravasation irritant [3]. Subcutaneous (SC) injections are only used for administering non-irritating drugs and result in slower absorption of some drugs, particularly vasoconstrictor agents [1]. Ulcerous skin lesions have been found to occur in mice and dogs after intradermal injection of 10 mg/ml carboplatin [10, 11]. In contrast, previous studies have demonstrated that SC injections of diluted carboplatin (300 mg/m^2) over 3–5 days [13], and the injection of slow-release materials have a high level of safety in dogs [8]. However, the serum concentration of carboplatin administered via SC injection has not yet been determined.

This study aimed to evaluate the serum concentrations of carboplatin administered via SC injections and compare them with that administered via an intravenous drip (IVD). The secondary aim was to describe any local or systemic adverse effects following carboplatin administration. We hypothesized that SC administration of diluted carboplatin would be safe and facilitate longer-lasting serum concentrations than that of carboplatin administered via a 30-min IVD.

MATERIALS AND METHODS

Three male and two female beagles that were bred for research purposes were used in this study. The median age and bodyweight of the dogs were 6 (range, 1–8) years and 11.4 (range 7.6–14.0) kg, respectively. The study protocol was approved

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by the Azabu University Animal Board (No. 150701-2). Carboplatin was administered via IVD and SC injection, and controlled crossover study evaluations were performed regularly to detect any side effects. The collected data included the clinical signs, physical examination findings, and blood test results.

Carboplatin administration

Carboplatin (carboplatin 450 mg, Sawai Pharmaceutical Co., Ltd., Osaka, Japan) was administered at a dose of 300 mg/m^2. For SC injection, carboplatin was diluted in 500 ml/m^2 of saline and administered over 5 min using a 21-G butterfly needle. For the IVD, an indwelling cannula (22 G, 1 inch, Terumo Corp., Tokyo, Japan) was inserted into the cephalic vein. Carboplatin was then administered using an infusion pump (TOP-221V, TOP Corp., Tokyo, Japan) for 30 min. Only a single dose of carboplatin was administered, after which the patients were monitored.

We employed a crossover design in this study. Three of the dogs initially received SC injections of carboplatin, while the other two dogs received an IVD of carboplatin. After a minimum of 3 weeks, each dog received the other treatment after confirming that the general condition and blood test results were normal.

Toxicity monitoring

Clinical signs, including any evidence of skin toxicity, were observed daily for 21 days after each carboplatin administration. Blood was collected before administration and at 7, 14, and 21 days after administration to assess the patient's complete blood counts and blood chemistry results. C-reactive protein (CRP) levels were measured 4 days after the administration of carboplatin.

Adverse events, including myelosuppression, renal and hepatic toxicity, clinical signs, and skin toxicity, were assessed using the veterinary cooperative oncology group-common terminology criteria for adverse events (VCOG-CTCAE) [14]. An area around the injection site with a 10-cm radius was observed for signs of skin toxicity.

Pharmacokinetic analysis of the serum carboplatin concentration

Blood collection for the measurement of serum carboplatin concentration after IVD administration (IVD group) was performed at 14 time points, that is, at 0 (just before administration), 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 3.5, 4.0, 5.0, and 6.0 hr after the initiation of carboplatin administration. Blood collection for the measurement of serum carboplatin concentration after SC administration (SC group) was performed at 10 time points, that is, 0 (just before administration), 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 hr after the initiation of carboplatin administration.

Serum concentrations were measured using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection (λ=230 nm, SPD-10A, Shimadzu Corp., Kyoto, Japan), comprising a pump (LC-10AS, Shimadzu Corp.), an autosampler (SIL-20A, Shimadzu Corp.), and a UV detector (SPD10A, Shimadzu Corp.). The analytical column was a C18 column (Triart C18, YMC Co., Ltd., Kyoto, Japan), and the effluent from the column was monitored using UV detection at a wavelength of 230 nm. The mobile phase consisted of 0.1 M KH_2PO_4 and 1 mM ethylenediaminetetraacetic acid at a flow rate of 0.6 ml/min. A 200-µl sample of serum was mixed with 400 µl of acetonitrile and centrifuged at 9,000 rpm for 10 min. The supernatant was removed, placed in a glass test tube, and evaporated using nitrogen gas. The residue was reconstituted with 400 µl of the mobile phase. A 100-µl sample was then measured using HPLC. The lower limit of quantification was 1 µg/ml, and the inter- and intra-day variations (1–30 µg/ml) were within 10%. We used the time course of the serum concentration of carboplatin to calculate the half-life. Furthermore, the area under the curve (AUC) of IVD and SC administration was calculated.

Statistical analysis

The VCOG grades of neutrophils and platelets were compared using Student's *t*-test for paired data. CRP levels were compared using a paired Student's *t*-test, and Bonferroni correction was performed to adjust for multiple comparisons. All statistical analyses were performed using JMP software (version 8.02; SAS Institute, Cary, NC, USA). A *P*-value of ≤0.05 was considered statistically significant.

RESULTS

No skin toxicity or abnormal clinical signs were observed in any of the dogs in this study. The blood test abnormalities are shown in Table 1. We detected decreased neutrophil and platelet counts. Minor elevations in CRP were observed in all dogs except for one dog whose CRP did not change in both the IVD and SC protocols compared to pre values, and changes greater than 1.0 mg/dl in these individuals were observed at 14 days post-dose (n=1; IVD: 4.6 mg/dl, n=1; SC: 1.35 mg/dl). Based on the VCOG scale, neutropenia can be classified as grades I, II, III, and IV. In the current study, IVD and SC administration of carboplatin caused grade I neutropenia in two and two dogs, grade II neutropenia in two and 0 dogs, grade III neutropenia in one and one dog, and grade IV neutropenia in 0 and one dog, respectively (Table 1). The nadir of the neutrophil count was detected on day 14 (n=9) and day 7 (n=1; IVD) after carboplatin administration (Fig. 1A). There was no significant difference in the neutropenia scores between the groups. Based on the VCOG scale, thrombocytopenia can be classified as grades I, II, III, and IV. In the current study, the IVD and SC administration of carboplatin caused grade I thrombocytopenia in two and 0 dogs, grade II thrombocytopenia in 0 and one dog, and grade III thrombocytopenia in 0 and one dog, respectively (Table 1). There was no significant difference in the scores between the groups. The nadir of the platelet count was detected on day 14 (n=8) and day 7 (n=2; SC) after carboplatin administration (Fig. 1B). There was no significant difference in CRP levels between the groups.

Table 1. Number of dogs diagnosed with neutropenia and thrombocytopenia

		Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia	IVD (n)	2	2	1	0
	SC (n)	2	0	1	1
Thrombocytopenia	IVD (n)	2	0	0	-
	SC (n)	0	1	1	-

Neutropenia and thrombocytopenia were classified according to the guidelines established by the VCOG-CTCAE. IVD, intravenous drip; SC, subcutaneous administration; VCOG-CTCAE, veterinary cooperative oncology group-common terminology criteria for adverse events.

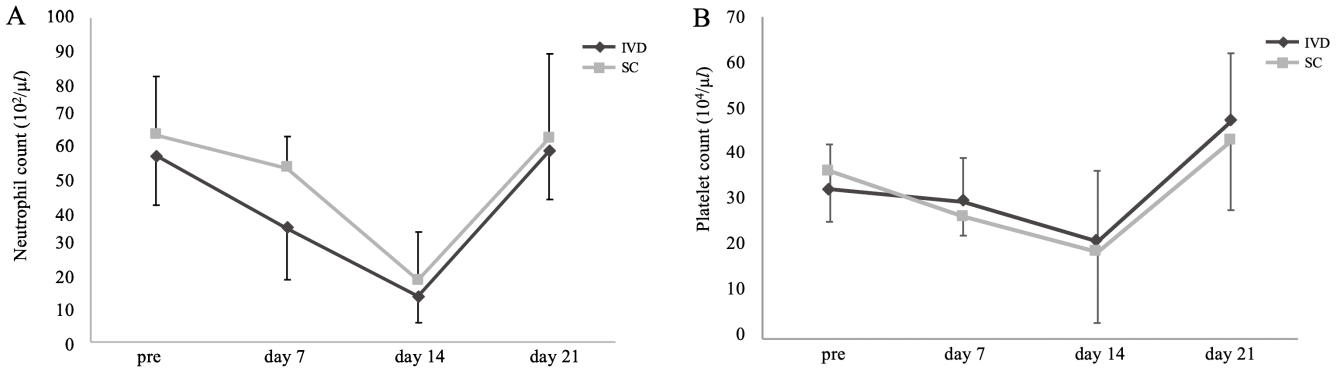

Fig. 1. The nadirs of the neutrophil (A) and platelet (B) counts in the intravenous drip (IVD) and subcutaneous (SC) groups over the 21 days following carboplatin administration. Data are presented as the mean ± standard deviation (SD).

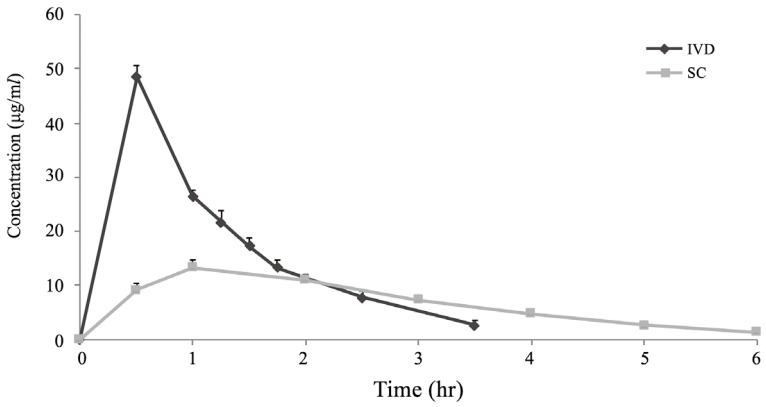

Fig. 2. The concentration versus time curve (mean ± standard deviation [SD]) of systemically absorbed carboplatin (μg/ml) after intravenous drip (IVD) and subcutaneous (SC) administration of carboplatin.

Table 2. Summary of the pharmacokinetic analysis of carboplatin (n=5)

	SC		IVD	
	Mean	SE	Mean	SE
Cmax (μg/ml)[a)]	13.7	1.3	49.0	1.7
Tmax (hr)[b)]	1.2	0.1	0.6	0.1
T1/2β (hr)[c)]	1.5	0.1	0.8	0.1
AUC (μg*hr/ml)[d)]	41.5	2.9	63.1	4.1
F (%)[e)]	66.5	4.6	ND	ND

[a)] Peak serum concentration, [b)] time of peak serum concentration, [c)] Elimination half-life, [d)] area under the blood concentration-time curve, [e)] bioavailability AUC, area under the curve; IVD, intravenous drip; ND, not detected; SC, subcutaneous.

The maximum serum carboplatin concentrations after IVD and SC administration were 49.0 ± 1.7 μg/ml and 13.7 ± 1.3 μg/ml, respectively (P<0.001). The time to maximum carboplatin concentration after IVD and SC administration was 0.55 ± 0.05 and 1.2 ± 0.12 hr, respectively (Fig. 2). There was a significant difference between the groups (P=0.012). The minimum serum carboplatin concentration (1.0 μg/ml) was detected 4 hr and 6 hr after the administration in the IVD and SC groups, respectively. The serum carboplatin half-lives were 0.8 ± 0.1 hr and 1.5 ± 0.1 hr for the IVD and SC groups, respectively. There was a significant difference between the groups (P=0.008). The AUCs of the IVD and SC groups were 63.1 ± 4.1 μg·hr/ml and 41.5 ± 2.9 μg·hr/ml, respectively (Table 2). There was a significant difference between the groups (P=0.002).

DISCUSSION

This study demonstrated three important findings concerning SC injections of carboplatin. First, local toxicity of SC carboplatin was not observed macroscopically in a single administration of carboplatin. The extravasation of carboplatin at concentrations ≥10 mg/ml is likely to cause ulcerations in mice and dogs [10, 11]. Moreover, in a study by Santamaria *et al.* involving 45 dogs, infections were reported at the surgical site in 22 dogs [13]. Additionally, these dogs received SC infusion of carboplatin 5–6 mg/ml (300 mg/m^2) for 3–7 days. Santamaria *et al.* suggested that the discharge may have been related to a sterile immunogenic reaction or direct tissue irritation by carboplatin [13]. In contrast, no skin toxicity was observed macroscopically up to 21 days after carboplatin administration in this study. CRP level was elevated to the upper normal limit; however, most of the cases were within normal limits. Therefore, it was not specific for SC administration. Our study demonstrated that a single SC injection of low concentration carboplatin (0.6 mg/ml) is safe for short-term use. However, the cumulative effects were not evaluated.

Second, the systemic toxicity of SC carboplatin administration was comparable to that of IVD administration. Concerning chemotherapy, myelosuppression is dose-limiting toxicity [15]. In a previous study, Santamaria *et al.* reported that four of 45 dogs receiving a continuous SC (300 mg/m^2) infusion of carboplatin had gastrointestinal side effects [13]. They also reported that the incidence of adverse bone marrow-related effects was similar to that reported in previous studies [4, 5, 12]. In this study, no gastrointestinal side effects occurred clinically, and the adverse events observed were similar in both groups. Therefore, our results suggest that safety of SC carboplatin is similar to that of IVD administration.

Third, the carboplatin serum peak concentrations and AUCs of IVD administration were superior to those of SC administration. Carboplatin has also been reported to show AUC-dependent efficacy [9]. However, the half-life of SC carboplatin administration was longer than that of IVD administration. The most important pharmacokinetic parameters are those that are related to a response to therapy or toxicity and are most often demonstrated by the AUC or the maximum drug concentration (C_{max}) achieved [15]. In contrast, carboplatin administered as an implant results in a slow release of the drug and has been reported to improve survival time for dogs with osteosarcoma [2, 8]. Furthermore, it was reported that the IC_{50} value (i.e., the drug concentration causing 50% inhibition of clonogenic survival) of carboplatin was 0.5–1.6 µg/ml in ovarian carcinoma cell lines. It is suggested that the subcutaneous administration may provide effective blood levels [6]. The results of these studies, along with the long-lasting blood concentrations of carboplatin observed in this study, suggest that the slow release of the drug may affect tumor control. Therefore, a longer half-life of carboplatin may be more effective for some tumors.

There were several limitations to this study. First, the number of cases was low and healthy beagle dogs were used. Therefore, prospective studies with a larger sample size that evaluate IVD versus SC carboplatin in pathological conditions are warranted. Second, since this study evaluated the adverse events of a single administration of carboplatin in healthy dogs, the effects of repeated administration in dogs will be a subject of future study as repeated exposure to local carboplatin might be affected by the skin in old dogs. Third, subclinical damage was not detected in this study design, and more sensitive tests should be adopted, particularly for skin and renal toxicity detection. Fourth, this study evaluated only adverse events. As a result of AUC, the antitumor effect may be reduced in SC carboplatin cases.

In conclusion, the results of this study demonstrate that carboplatin remains in the serum longer after SC administration than after IVD administration. However, the AUC was less with SC than with IVD administration. The systemic toxicity observed with SC carboplatin was comparable to that of IVD carboplatin, and no gross skin damage was not detected, and abnormal clinical signs were not observed in any of the dogs. Therefore, the results of this study suggest that SC carboplatin may be efficacious in the treatment of tumors in dogs, particularly those in which IVD administration is challenging.

CONFLICT OF INTEREST. The authors declare no conflicts of interest.

REFERENCES

1. Barnes, C. D. and Eltherington, L. G. 1966. Drug Dosage in Laboratory Animals. A Handbook. University of California Press, Berkeley.
2. Bergman, P. J., MacEwen, E. G., Kurzman, I. D., Henry, C. J., Hammer, A. S., Knapp, D. W., Hale, A., Kruth, S. A., Klein, M. K., Klausner, J., Norris, A. M., McCaw, D., Straw, R. C. and Withrow, S. J. 1996. Amputation and carboplatin for treatment of dogs with osteosarcoma: 48 cases (1991 to 1993). *J. Vet. Intern. Med.* **10**: 76–81. [Medline] [CrossRef]
3. Biller, B., Berg, J., Garrett, L., Ruslander, D., Wearing, R., Abbott, B., Patel, M., Smith, D. and Bryan, C. 2016. AAHA oncology guidelines for dogs and cats. *J. Am. Anim. Hosp. Assoc.* **52**: 181–204. [Medline] [CrossRef]
4. Boria, P. A., Glickman, N. W., Schmidt, B. R., Widmer, W. R., Mutsaers, A. J., Adams, L. G., Snyder, P. W., DiBernardi, L., de Gortari, A. E., Bonney, P. L. and Knapp, D. W. 2005. Carboplatin and piroxicam therapy in 31 dogs with transitional cell carcinoma of the urinary bladder. *Vet. Comp. Oncol.* **3**: 73–80. [Medline] [CrossRef]
5. Chun, R., Knapp, D. W., Widmer, W. R., DeNicola, D. B., Glickman, N. W., Kuczek, T., Degortari, A. and Han, C. M. 1997. Phase II clinical trial of carboplatin in canine transitional cell carcinoma of the urinary bladder. *J. Vet. Intern. Med.* **11**: 279–283. [Medline] [CrossRef]
6. Engblom, P., Rantanen, V., Kulmala, J. and Grènman, S. 1999. Carboplatin-paclitaxel- and carboplatin-docetaxel-induced cytotoxic effect in epithelial ovarian carcinoma in vitro. *Cancer* **86**: 2066–2073. [Medline] [CrossRef]
7. Goolsby, T. V. and Lombardo, F. A. 2006. Extravasation of chemotherapeutic agents: prevention and treatment. *Semin. Oncol.* **33**: 139–143. [Medline] [CrossRef]
8. Hess, T. A., Drinkhouse, M. E., Prey, J. D., Miller, J. M., Fettig, A. A., Carberry, C. A., Brenn, S. H. and Bailey, D. B. 2018. Analysis of platinum content in biodegradable carboplatin-impregnated beads and retrospective assessment of tolerability for intralesional use of the beads in dogs following excision of subcutaneous sarcomas: 29 cases (2011–2014). *J. Am. Vet. Med. Assoc.* **252**: 448–456. [Medline] [CrossRef]

9. Jodrell, D. I., Egorin, M. J., Canetta, R. M., Langenberg, P., Goldbloom, E. P., Burroughs, J. N., Goodlow, J. L., Tan, S. and Wiltshaw, E. 1992. Relationships between carboplatin exposure and tumor response and toxicity in patients with ovarian cancer. *J. Clin. Oncol.* **10**: 520–528. [Medline] [CrossRef]
10. Marnocha, R. S. M. and Hutson, P. R. 1992. Intradermal carboplatin and ifosfamide extravasation in the mouse. *Cancer* **70**: 850–853. [Medline] [CrossRef]
11. Miller, K. B., Lejeune, A., Regan, R., Szivek, A. and Kow, K. 2018. Suspected carboplatin extravasation reactions in seven dogs. *J. Am. Anim. Hosp. Assoc.* **54**: 360–367. [Medline] [CrossRef]
12. Rassnick, K. M., Ruslander, D. M., Cotter, S. M., Al-Sarraf, R., Bruyette, D. S., Gamblin, R. M., Meleo, K. A. and Moore, A. S. 2001. Use of carboplatin for treatment of dogs with malignant melanoma: 27 cases (1989–2000). *J. Am. Vet. Med. Assoc.* **218**: 1444–1448. [Medline] [CrossRef]
13. Santamaria, A. C., Simcock, J. O. and Kuntz, C. A. 2019. Adverse events and outcomes in dogs with appendicular osteosarcoma treated with limb amputation and a single subcutaneous infusion of carboplatin. *J. Am. Vet. Med. Assoc.* **255**: 345–351. [Medline] [CrossRef]
14. Veterinary cooperative oncology group (VCOG). 2016. Veterinary cooperative oncology group - common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1. *Vet. Comp. Oncol.* **14**: 417–446. [Medline]
15. Vail, D. M. 2012. Pharmacologic Principles in Cancer. pp. 163–165. In: Withrow & MacEwen's Small Animal Clinical Oncology, 5th ed. (Withrow, S. J., Vail, D. M. and Page, R. eds.), Elsevier Saunders, Philadelphia.