Cancer Chemother Pharmacol (2008) 62:545–549
DOI 10.1007/s00280-007-0615-4

SHORT COMMUNICATION

Influence of carboplatin infusion on osteosarcoma blood flow

Gene R. DiResta · Sean W. Aiken · Holly K. Brown · Philip J. Bergman · Ann Hohenhaus · John H. Healey

Received: 18 April 2007 / Accepted: 19 September 2007 / Published online: 12 October 2007

Abstract
Purpose Herein we report that carboplatin infusion influenced tumor blood flow signal independent of the mechanical decompression induced by the artificial lymphatics system technology that was being evaluated as part of a randomized veterinary clinical trial, treating spontaneously occurring canine appendicular osteosarcoma, a tumor very similar to its human counterpart.
Methods Blood flow within the central region of the tumor was recorded continuously using laser Doppler flowmetry, a real-time measurement technology. Time-averaged flow values were computed from segments taken from the recordings immediately before starting carboplatin infusion, and during infusion.
Results Carboplatin increased the tumor blood flow signal by an additional 59 ± 26% (mean ± SEM; $p = 0.06$) over the increase induced by the decompression. The increase started within 49 ± 46 s after the start of infusion, had a response time constant of 19 ± 21 s and persisted throughout the infusion, ending shortly after infusion ended.
Conclusion The rapidity of the flow signal increase suggests that carboplatin may have an autonomic effect on circulation, either local or systemic. The observations identify a new action of this drug and suggest a possible mechanism to exploit therapeutically.

Keywords Canine · Osteosarcoma · Artificial lymphatic system · Carboplatin · Blood flow · Interstitial fluid pressure

G. R. DiResta (✉) · H. K. Brown · J. H. Healey
Memorial Sloan Kettering Cancer Center, Department of Surgery, Associated with Weill Medical College of Cornell University, Orthopaedic Surgery Service, 1275 York Avenue, New York, NY 10021-6007, USA
e-mail: direstag@mskcc.org

S. W. Aiken · P. J. Bergman · A. Hohenhaus
The Animal Medical Center, Donaldson-Atwood Cancer Clinic, 510 E 62nd Street, New York, NY 10021, USA

P. J. Bergman
Flaherty Comparative Oncology Laboratory, The Animal Medical Center, 510 E 62nd Street, New York, NY 10021, USA

Introduction

Carboplatin, a chemotherapeutic agent for treating solid tumors [1], was used in a veterinary clinical trial performed at The Animal Medical Center, New York to investigate the efficacy of an "artificial lymphatic system" (ALS) to enhance the drug's uptake into spontaneously-occurring, canine appendicular osteosarcoma (OS) [3]. The ALS is a new local treatment adjunct technology developed by Memorial Sloan-Kettering Cancer Center to reduce tumor interstitial hypertension and increase blood flow within solid tumors [4] during chemotherapy or radiotherapy administration. The technique involves the placement of aspiration drains within the tumor and applying –80 mmHg vacuum pressure to remove the fluid associated with the elevated interstitial fluid pressure observed in solid tumors [4]. The net effect of ALS decrease in intratumoral interstitial pressure is to increase blood flow through the tumors' viable capillary network surrounding the drain during the aspiration period. The increased blood flow increases the delivery of therapeutic agents and pO_2 levels in tissue regions influenced by the aspiration [3–5]. The use of an ALS, though a mechanical treatment adjunct, is more predictable and reproducible than the use of vasoactive drugs [3] for

increasing local tumor blood flow. During the trial, the ALS' temporal effects on tumor capillary blood flow were continuously measured using laser Doppler flowmetry (LDF), a flow measurement technology whose temporal response is capable of quantifying capillary blood flow transients in real-time, although within small tissue volumes [4]. Herein we report from that clinical trial, the unanticipated influence of carboplatin to increase the OS' LDF signal.

Methods

Large breed dogs, with histologically confirmed appendicular osteosarcoma, were recruited into a veterinary clinical trial performed at The Animal Medical Center, New York. All procedures used in the trial were approved by the IACUC of The Animal Medical Center, and Memorial Sloan-Kettering Cancer Center. The surgical and measurement details are presented in DiResta et al [3]. Every dog was mechanically ventilated, and received a 0.9% saline drip throughout the entire surgical procedure to control rate and depth of respiration, and compensate for fluid loss, respectively. Briefly, tumor capillary blood flow was measured using LDF within a $\sim$1 mm^3 volume adjacent to a single ALS drain positioned in the tumor center prior to initiating –80 mmHg vacuum to the drain, during vacuum application; and during carboplatin infusion. LDF was used to measure the tumors' blood flow changes because its temporal response time, $\sim$0.1 s [4], is faster than the tumors' flow changes following ALS drainage, i.e. 2 s–2 min [3]. Systemic carboplatin (300 mg/m^2) infusion was started when the LDF signal stabilized, typically 10–20 min after vacuum application. The drug was added to 125 ml of 5% dextrose. The pump rate was adjusted such that each dog's infusion time was 10 min. LDF and electrocardiogram (ECG) were recorded continuously during the entire study. Figure 1a displays the timing used in the study, where, M1–M5 are the recording segments that were tabulated at the conclusion of the study using Excel XP (Microsoft, Inc., USA). Each interval was chosen from "flat" regions of the LDF recording. This work presents the "pre-carbo infusion" (M3) and "during-carbo infusion" (M4) LDF values. M1, M2 and M3 relate to the infuence of the ALS treatment on blood flow. These findings are presented in DiResta et al. [3]. The OS tissue was then biopsied to obtain tissue for carboplatin content and the dog's limb was amputated.

Results

The "pre-carbo infusion" and "during-carbo infusion" blood flow values for the 12 dogs where tumor blood flow was successfully recorded are presented in Fig. 1b. The "pre-carbo infusion" interval was 24 ± 7 s (mean ± SEM). However, the"during-carbo infusion" interval was 100 ± 63 s (mean ± SEM) because larger amplitude changes were observed in the LDF recording during carboplatin infusion. Carboplatin increased flow in eight dogs, had no effect on two dogs and decreased flow in the other two dogs. Using the dog 1–8 data, tumor blood flow increased 59 ± 26% (mean ± SEM; $p = 0.06$) over "pre-carbo infusion" levels. Figure 2 is the annotated LDF data recording from dog 2 whose flow signal rapidly increased following administration of carboplatin. The influence appears to have a rapid initial component that then drops to a stable level. In this dog, the time constant for the initial component is 1.5 s^{-1}. The ECG signal is included and shows an unexplained periodic dip during infusion. The drug's effect reversed approximately 8 min after infusion ended.

The time constant ($t_{1/2}$) and delay (t_{lag}) associated with the LDF signal changes following carboplatin infusion were estimated using OriginPro v7.5 (Origin Labs, Northampton, MA, USA) with the equation:

$$F_{\text{ldf}} = F_0 + F_1 \times \left(1 - e^{\left(-\left(t - t_{\text{lag}}\right) \times \ln(2)/t_{1/2}\right)}\right)$$

F_{ldf} is the recorded LDF signal, F_0 is the signal when carboplatin infusion began, and F_1 is the signal increase associated with the carboplatin effect. In some cases, e.g., Fig. 2, F_1 remained elevated for 30 sec and then decreased to a lower stable value throughout the infusion period. For the estimation of $t_{1/2}$, only the higher F_1 was used to reflect the rapid initial response of the LDF signal to the carboplatin infusion. The delay parameter, t_{lag}, estimates the time duration from the start of carboplatin infusion to the time the signal increase begins and includes the fluid transit time from the carboplatin reservoir to the indwelling venous catheter. Figure 3, a box plot, presents the parameter value clusters for $t_{1/2}$ (19 ± 21 s; $n = 8$) and t_{lag} (49 ± 46 s; $n = 7$). The infusion start time for the first case was not recorded because an effect was not anticipated.

Discussion

The clinical trial demonstrated that ALS use increased local tumor blood flow and uptake of carboplatin [3], corroborating controlled rat tumor studies [5]. The carboplatin-induced increase in tumor blood flow has not been reported. The LDF recordings suggest that its effect is rapid, beginning in our study on an average of 49 s after the drug infusion began and lasting several minutes after infusion ended. When the fluid delivery transit time ($\sim$30–

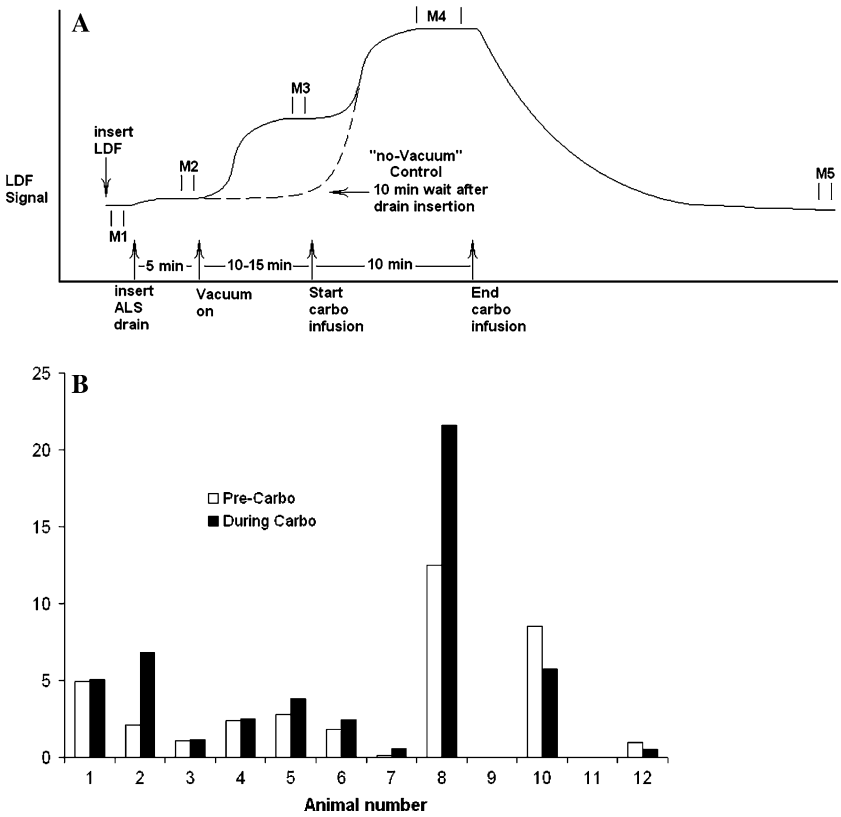

Fig. 1 **a** The timing diagram used in the clinical ALS study showing the time interval and sampling location; where, LDF was time-averaged for tabulation. **b** Blood flow before, and during carboplatin infusion in the 12 study dogs

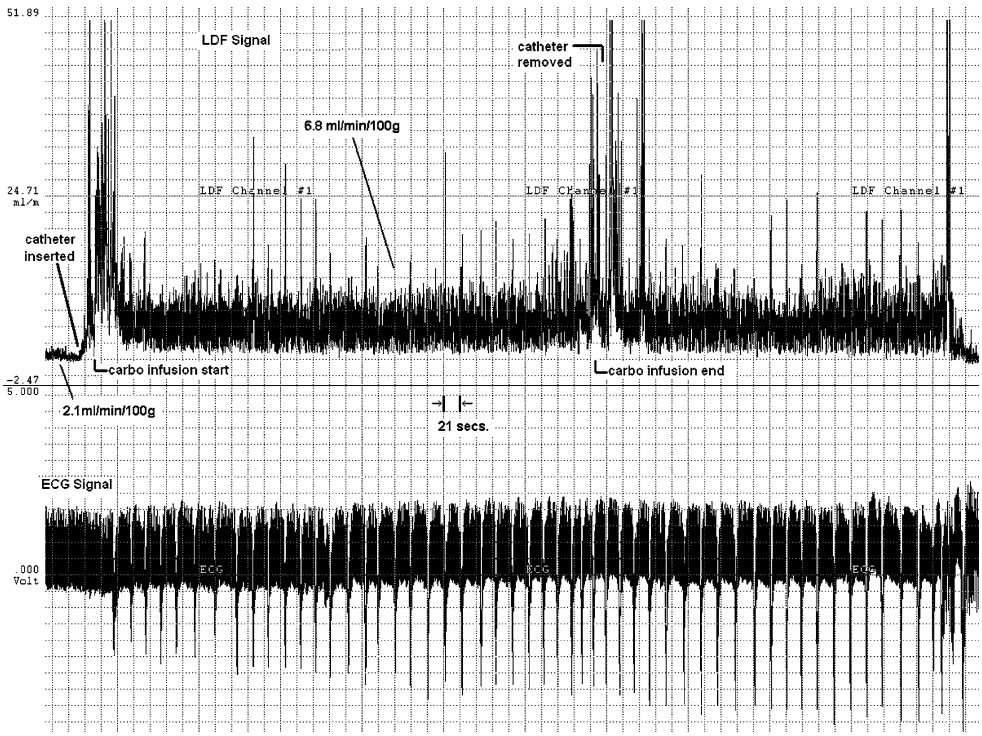

Fig. 2 Section of raw data recording of dog 2 showing the response of LDF and ECG to carboplatin infusion

60 s) is removed from t_{lag}, the carboplatin response delay ($\sim$15–30 s) is attributed to its venous transit and physiologic effect. The short delay time and rapidity of the response, i.e. 19 s, suggest that the drug may have an autonomic effect on circulation, either local or systemic. Furthermore the rapid changes in F_1 suggest that the dog is able to partly compensate for the drug's initial effect. Thus a measurement technology with a fast temporal response

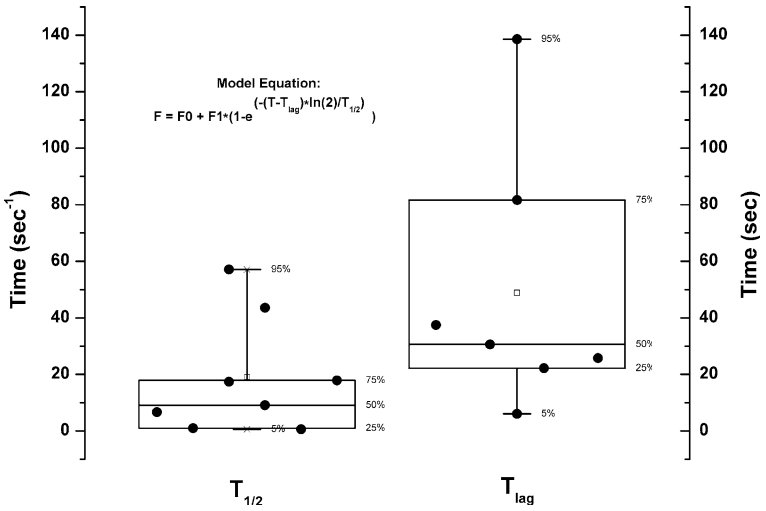

Fig. 3 Box chart of time constant and time lag for the eight dogs whose blood flow signal increased during infusion of carboplatin

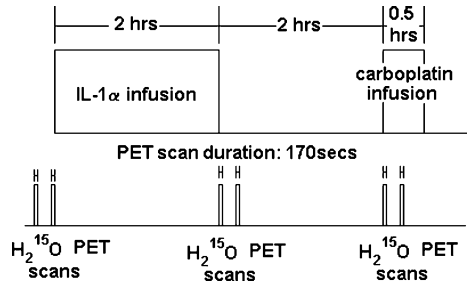

Fig. 4 Timing diagram prepared using information presented in Logan et al. study [6]

such as LDF is required to evaluate the sequence of the response.

During the administration of cisplatinum (2 mg/kg), Chapman and Horseman report a 20–30% increase in tumor blood flow in SCC VII tumors grown in the hind foot dorsum of C_3H/He mice. Drug infusion was via indwelling catheter in the mouse tail vein. Blood flow measurements were obtained using laser Doppler flowmetry. From their Fig. 6, flow increase began approximately 1 min after the infusion began. In contrast, tumor blood flow decreased following infusion of hypoxic cell cytoxin RSU 1069; pimonidazole Ro-03-8799; and doxorubicin.

Logan et al performed a human phase I clinical trial to investigate the influence of interleukin-1 (IL-1(α) on carboplatin pharmacokinetics and tumor blood flow. The patient tumors were all metastatic lung lesions whose primary included lung, renal, breast, melanoma, thyroid, and colorectal cancers. $H_2^{15}O$-positron emission tomography (PET) was the modality used to measure tumor blood flow. The timing diagram shown in Fig. 4 prepared using information from their "Methods" section illustrates the temporal relationships between their drug administration protocol with the timings of PET measurements.

They reported a statistically significant decrease ($p < 0.008$) in tumor blood flow following IL-1α infusion. However, following the infusion of carboplatin, tumor blood flow significantly increased relative to the measures obtained at the conclusion of the IL-1α infusion to levels that were indistinguishable from pre IL-1α infusion values ($p = 0.25$). From their analysis of the patients' cardiovascular parameters, e.g., blood pressure, they concluded that the temporal flow changes associated with drug infusion were not solely associated with hypotensive effects, but offered no alternative explanation for the flow increase.

On the basis of our canine observations with carboplatin, and the mouse observations with cisplatinum, Logan et al's reported an increase in tumor blood flow following carboplatin infusion may be attributed to a neurogenic influence of carboplatin, but the phenomena requires further investigation. The $H_2^{15}O$-PET flow measurement modality required 2–3 min to perform and as such does not have the temporal flow resolution of LDF. However, as suggested by the canine LDF observations and the timing diagram, the PET measures occurred during the interval that the tumor blood flow may have been influenced by carboplatin infusion. The observations in the naturally occurring canine and human tumor studies, though coincidental and anecdotal, suggest that carboplatin may directly increase the blood flow as was observed with cisplatinum in the controlled mouse study. Rat studies have demonstrated that increasing tumor blood flow following ALS decompression also increases local tumor pO_2[4]. Therefore, if the flow enhancing effect of these two platinum drugs is confirmed, their influence may be therapeutically useful for rapid therapies such as external beam radiation, where an increased flow may increase local pO_2, a potent radiation sensitizing agent [2], while simultaneously contributing a chemotherapeutic effect.

Acknowledgments This research was supported in part by NIH grant No. RO1-CA7884494-01A1, and the Yurman, and Perlman Limb Preservation Funds.

References

1. Bergman PJ, MacEwen EG, Kurzman ID, Henry CJ, Hammer AS, Knapp DW, Hale A, Kruth SA, Klein MK, Klausner J, Norris AM, McCaw D, Straw RC, Withrow SJ (1996) Amputation and carboplatin for treatment of dogs with osteosarcoma: 48 cases (1991–1993). J Vet Intern Med 10(2):76–81
2. Chaplin DJ, Horseman MR (1992) Tumor blood flow changes induced by chemical modifiers of radiation response. Int J Rad Onc Biol Phys 22(3):459–462
3. DiResta GR, Aiken SA, Brown HK, Bergman PJ, Hohenhaus A, Ehrhart EJ, Baer K, Healey JH (2007) Use of an artificial lymphatic system during carboplatin infusion to improve canine osteosarcoma blood flow and clinical response. Ann Surg Onc 14(8):2411–21
4. DiResta GR, Lee J, Healey JH, Levchenko A, Larson SM, Arbit E (2000) The artificial lymphatic system: a new approach to reduce interstitial hypertension and increase blood flow, pH, and pO_2 in solid tumors. Ann Biomed Eng 28(5):543–555
5. DiResta GR, Lee J, Healey JH, Larson SM, Arbit E (2000) Enhancing the uptake of chemotherapeutic drugs into tumors using an artificial lymphatic system. Ann Biomed Eng 28(5):556–564
6. Logan TF, Jadali F, Egorin MJ, Mintun M, Sashin D, Gooding WE, Choi Y, Bishop H, Trump DL, Gardner D, Kirkwood J, Vlock D, Johnson C (2002) Decreased tumor blood flow as measured by positron emission tomography in cancer patients treated with interleukin-1 and carboplatin on a phase I trial. Cancer Chemother Pharmacol 50:433–444