Published in final edited form as:

Vet Comp Oncol. 2014 June ; 12(2): 114–119. doi:10.1111/j.1476-5829.2012.00340.x.

Development of a limited-sampling model for prediction of doxorubicin exposure in dogs

L. A. Wittenburg¹, D. H. Thamm^{1,2}, and D. L. Gustafson^{1,2}

¹Department of Clinical Sciences, Colorado State University Animal Cancer Center, Fort Collins, CO, USA

²University of Colorado Comprehensive Cancer Center, Anschutz Medical Campus, Aurora, CO, USA

Abstract

Understanding the relationship between drug dose and exposure (pharmacokinetics, PK) and the relationship between exposure and effect (pharmacodynamics) is an important component of pharmacology when attempting to predict clinical effects of anticancer drugs. PK studies can provide a better understanding of these relationships; however, they often involve intensive sampling over an extended period of time, resulting in increased cost and decreased compliance. Doxorubicin (DOX), one of the most widely used antineoplastic agents in veterinary cancer therapy, is characterized by large interpatient variability in overall drug exposure and the development and degree of myelosuppression following equivalent dosages. We have developed and validated a limited-sampling strategy for DOX, in which three blood samples are obtained over 1 h post-treatment, that accurately predicts patient exposure. This strategy could allow for refining of dosing variables and utilization of therapeutic drug monitoring to ensure optimized dosing.

Keywords

adriamycin; canine; limited sampling; pharmacodynamics; pharmacokinetics

Introduction

Doxorubicin (DOX), one of the most commonly used antineoplastic agent in veterinary cancer therapy, is characterized by a narrow therapeutic window, with only small differences in drug exposure resulting in either reduced efficacy or increased toxicity, and large variability in exposure between patients following equivalent doses. Understanding the relationship between drug dose and exposure (pharmacokinetics, PK) and the relationship between exposure and efficacy or toxicity (pharmacodynamics, PD) is an important component of cancer pharmacology when attempting to predict the clinical effects of anticancer drugs. Despite evidence demonstrating that there is a strong correlation between patient exposure to antineoplastic drugs and toxicities such as bone marrow suppression, the majority of these drugs are still dosed based on the body surface area (BSA) of the patient. Dosing based on a patient's BSA was initially instituted when it was found that this measure of dosing could normalize the maximum tolerated dose of chemotherapeutic drugs in mice,

rats, dogs and humans.¹ This was suggested to be due to the fact that BSA correlates better with physiologic processes and functions that influence drug activity in the body than weight¹; however, it has been demonstrated that there is increased toxicity in smaller dogs when dosing on a BSA basis with a number of antineoplastic drugs,^{2–5} suggesting that either the current formula used to estimate BSA or the assumption that BSA correlates with antineoplastic drug exposure is inaccurate. In fact, the reason that BSA dosing in dogs does not always normalize drug exposure and toxicity may be explained by the fact that the current BSA dosing formula was validated based on differences in metabolic rate among species, not animals of different breeds within a species. There is evidence that the interspecies formula for correlating body size with metabolic rate is significantly different from the intraspecies formula.⁶ In addition, the formula for calculating BSA from body weight in dogs [$BSA = K \cdot (\text{weight})^{2/3}$], where K is a shape constant for the species, ignores the vast differences in shape and conformation among different breeds of dogs.⁷

Although it is clear that there are some instances where BSA dosing is appropriate, these dosing regimens do not take into consideration the effect of disease state on drug disposition. All the factors that contribute to the overall exposure to a drug (absorption for oral drugs, distribution, metabolism and excretion) have been shown to be affected by disease states such as ascites, pleural effusion and cachexia resulting in reduced plasma protein content. These conditions reduce any correlation between drug PK and BSA, again resulting in unpredictable variability in exposure and toxicity.

Given the information above, it is not surprising that reports in human oncology have found that drug exposure [area under the plasma concentration–time curve (AUC) or steady-state concentration] correlates more closely with PD effects than the dose per unit of BSA.^{8–11} In particular, studies in human patients administered DOX have demonstrated a lack of correlation between the dose administered and exposure (AUC), but a significant correlation between AUC and bone marrow suppression.^{11,12} The same has been found with methotrexate, where the AUC following high-dose therapy is significantly correlated with degree of neutropenia, better event-free survival and overall survival.¹⁰ A recent study in patients with metastatic colorectal cancer treated with fluorouracil (FU) compared conventional BSA-based dosing and PK-guided dose adjustment in terms of response, tolerability and survival and reported a significantly improved objective response rate, a trend towards higher survival rates, and less severe toxicity in the PK-guided group.¹³ It is important to note that in this same comparative study, the mean FU dose throughout treatment was higher in the PK-guided dosing group, whereas the adverse effects were significantly less frequent and severe, again illustrating that patient exposure correlates better with effect than does dose on a BSA basis. In addition, PK-guided dosing in human cancer therapy has also been shown to reduce interindividual variability in the degree of bone marrow suppression following docetaxel therapy by up to 50%.¹⁴

Current dosing regimens in veterinary oncology might be improved by implementing a strategy that utilizes individual exposure as a determinant of dose, based on an exposure–effect relationship. The first requirement in evaluating the relationship between drug exposure and effect is an ability to reliably measure exposure. Methods for the determination of PK parameters in dogs following DOX administration have previously been described and utilized clinically, but are laborious in that they require up to 10 samples collected over a 24-h period.^{15,16} The relatively high cost and reluctance of owners to enrol their pets in such studies can limit the utility of this approach in veterinary oncology. The development of a limited-sampling strategy for DOX, which would utilize only a few samples collected shortly after drug administration to predict overall exposure, would provide the opportunity to initiate studies that relate exposure to toxicity and efficacy in a large number of dogs.

Methods and results

The DOX serum concentration versus time data for 27 dogs treated at a dose of 30 mg m⁻² by 20-min infusion were utilized for calculation of drug exposure (AUC) by non-compartmental methods. The resulting AUC values were analysed as a response to timepoint DOX concentration values as a predictor by single and best subset multiple linear regression. This method evaluates all single timepoints as well as all possible combinations of multiple timepoints (2, 3, 4, 5, 6, 7 or 8 timepoints) as predictors of the outcome (AUC). The combinations of statistical correlation, number of samples required and length of time required post-infusion were all considered in choosing the optimal limited-sampling scheme. Patient population, sampling and analysis of the samples used in these studies have been previously described.¹⁶ Data from a total of 27 dogs with samples collected on day 1 of cycle 1 were utilized. Data utilized in the multiple linear regression analysis were only those timepoints corresponding to post-end-of-infusion (20 min) samples and were designated as 5, 15, 30, 45, 60, 180, 240 and 360, corresponding to the number of minutes the samples were collected after the end of drug infusion. All regression analysis was carried out using Minitab® v 15.1.1.0 software (Minitab, State College, PA, USA). The PK parameter utilized as an indicator of DOX exposure was AUC_{0→6 h} as determined by non-compartmental analysis. The basic model structure is described by the equation:

$$AUC = A + \sum_{i=1}^k B_i C_{\text{serum}}(t_i)$$

The results of best subset multiple linear regression revealed that using three timepoints as predictors of AUC could provide the best combination of statistical correlation while minimizing sample number and time post-infusion. Multiple linear regression was then performed using all combinations of DOX serum concentrations at time(s) t_1 , t_2 and/or t_3 as predictor variable(s) versus AUC_{0→6 h} as the response (Table 1). The combination of 5, 45 and 60 min post-end-of-infusion DOX serum concentrations correlated best with an r^2 of 91.9% (Fig. 1). Increasing the number of variables (timepoints) to four, five, six, seven or eight only increased the correlation by approximately 3% and required sampling out to 6 h. The final model utilizing the three identified timepoints is described by the equation:

$$AUC_{0 \rightarrow 6 \text{ h}} (\text{nM h}) = 46.9 + 0.63(C_{5 \text{ min}}) + 1.96(C_{45 \text{ min}}) + 6.63(C_{60 \text{ min}})$$

Following the identification of this limited-sampling set (5, 45 and 60 min) as the optimal predictor of AUC, three randomly generated training sets of 15 dogs were independently used with the remaining 12 dogs serving as a validation set. Validation of the proposed limited-sampling scheme was carried out using three independent cross-validations. The independent sets of 15 training samples were chosen using a web-based random number generator (www.random.org). Each set of 15 was used to generate an independent multiple linear regression model and the remaining 12 used as a test set. The three independent sets correlated with r^2 values of 92.8, 92.1 and 92.0% (Fig. 2). Analysis of the validation sets for AUC estimation showed median prediction errors ranging from 3.3 to 17.8% and absolute median prediction errors ranging from 6.2 to 17.8% (Table 2). Therefore, DOX exposure can be accurately predicted as measured by AUC_{0→6 h} using a limited-sampling scheme of serum samples collected at 5, 45 and 60 min post-end of 20-min infusion following a 30 mg m⁻² dose.

Discussion

With the clear lack of direct correlation between the dose of an antineoplastic drug administered on the basis of BSA and the efficacy or toxicity encountered, improvements in dosing strategies will require a better understanding of the cause of PD variability and the relationship to individual patient PK. Although some of the variability in toxicity and response to antineoplastic drugs in dogs is likely due to inaccuracies in the formula used to calculate BSA, it may also be due to the fact that size-independent factors have a greater influence on drug distribution, metabolism and excretion than do differences in body size. Several factors contribute to the overall drug concentration following an administered dose including absorption from the site of administration, distribution and storage in tissues, metabolism (both enzymatic and non-enzymatic) and excretion. All these processes would have to be correlated to BSA in order for this method of dosing to normalize exposure and toxicity in all cases. Metabolism represents an area of drug disposition that contributes greatly to the variability in toxicity of chemotherapeutics dosed by BSA. DOX is primarily metabolized by the enzyme aldo-keto reductase to form doxorubicinol in dogs.¹⁵ This enzyme has different affinity (K_m) and maximum rates (V_{max}) for the substrate in different species and tissues.¹⁷ The lack of correlation between metabolism of DOX and body size is illustrated by the fact that the affinity of the human form of aldo-keto reductase is greater than that of the dog, whereas the maximum rate of reductase activity in the dog exceeds that in humans.¹⁷ In addition to enzymatic metabolism, many chemotherapeutic drugs and their metabolites undergo non-enzymatic degradation, and there is no evidence that the rates of these non-enzymatic degradation processes are correlated to BSA.⁸

The concept of PK-guided dosing in cancer chemotherapy, which is essentially a method of therapeutic drug monitoring, seems logical because of the narrow therapeutic window and substantial interindividual variability seen with antineoplastic drugs. Although this approach has demonstrated improvements in some cases over BSA-based dosing in human cancer therapy, it has not been widely adopted for many antineoplastic drugs because the relationship between patient exposure and effect has not been completely elucidated for all drugs.^{18,19} The relationships between antineoplastic drug exposure and effect are even less well understood in veterinary medicine. Although there are studies that have correlated toxicity with patient outcome, particularly in canine lymphoma, they have not evaluated the relationship of drug exposure in the same setting.^{20–22} One conclusion that might be drawn from studies reporting prolonged survival in canine lymphoma patients that develop grade III or IV neutropenia following chemotherapy is that these patients actually have optimal drug exposure. However, the typical criteria for dose adjustments in veterinary medicine involve a reduction in dose of 20% for any drug resulting in grade III or IV neutropenia, and these reductions are generally permanently instituted in order to avoid future toxicity.²⁰ In addition, it is not typical for any dose increases to be made in patients that do not develop toxicity at standard doses. Sorenmo *et al.* have also suggested the importance of dose density in canine lymphoma, but with adjustment for the individual patient based on their own maximal tolerance.²² Without a clear relationship between dose and exposure, this would be a difficult undertaking because of the inherent variability in toxicity. However, if the relationship between exposure and toxicity was clearly defined for each patient following the first treatment cycle, the ability to dose to a 'target exposure' based on the predicted degree of toxicity becomes possible. However, potential limitations in the development of this sampling scheme might be in the inference that the same timepoints will be optimal for all dogs receiving DOX; the data utilized in this study were obtained from only 28 dogs during a single clinical trial and as such all dogs had the same limitations in regard to additional therapies and allowable comedications, which could have an effect on DOX PK.

This novel limited-sampling scheme for prediction of DOX exposure will be utilized to carry out PK/PD evaluation of the relationship between DOX exposure and degree of myelosuppression and gastrointestinal toxicity scores in a dose-escalation-based, multidrug canine lymphoma study in order to better understand how to refine dosing variables and utilize therapeutic drug monitoring to ensure optimized dosing with this important antineoplastic agent in veterinary oncology.

References

1. Pinkel D. The use of body surface area as a criterion of drug dosage in cancer chemotherapy. *Cancer Research*. 1958; 18:853–856. [PubMed: 13573353]
2. Page RL, Macy DW, Thrall DE, Dewhirst MW, Allen SL, Heidner GL, Sim DA, McGee ML, Gillette EL. Unexpected toxicity associated with use of body surface area for dosing melphalan in the dog. *Cancer Research*. 1988; 48:288–290. [PubMed: 3335006]
3. Arrington KA, Legendre AM, Tabeling GS, Frazier DL. Comparison of body surface area-based and weight-based dosage protocols for doxorubicin administration in dogs. *American Journal of Veterinary Research*. 1994; 55:1587–1592. [PubMed: 7879983]
4. Ogilvie GK, Richardson RC, Curtis CR, Withrow SJ, Reynolds HA, Norris AM, Henderson RA, Klausner JS, Fowler JD, McCaw D. Acute and short-term toxicoses associated with the administration of doxorubicin to dogs with malignant tumors. *Journal of the American Veterinary Medical Association*. 1989; 195:1584–1587. [PubMed: 2599942]
5. Ogilvie GK, Moore AS, Curtis CR. Evaluation of cisplatin-induced emesis in dogs with malignant neoplasia: 115 cases (1984–1987). *Journal of the American Veterinary Medical Association*. 1989; 195:1399–1403. [PubMed: 2584105]
6. Heusner AA. Energy metabolism and body size. I. Is the 0.75 mass exponent of Kleiber's equation a statistical artifact? *Respiratory Physiology*. 1982; 48:1–12.
7. Price GS, Frazier DL. Use of body surface area (BSA)-based dosages to calculate chemotherapeutic drug dose in dogs: I. Potential problems with current BSA formulae. *Journal of Veterinary Internal Medicine*. 1998; 12:267–271. [PubMed: 9686386]
8. van den Bongard HJ, Mathot RA, Beijnen JH, Schellens JH. Pharmacokinetically guided administration of chemotherapeutic agents. *Clinical Pharmacokinetics*. 2000; 39:345–367. [PubMed: 11108434]
9. Loh GW, Ting LS, Ensom MH. A systematic review of limited sampling strategies for platinum agents used in cancer chemotherapy. *Clinical Pharmacokinetics*. 2007; 46:471–494. [PubMed: 17518507]
10. Joerger M, Huitema AD, Krahenbuhl S, Schellens JH, Cerny T, Reni M, Zucca E, Cavalli F, Ferreri AJM. Methotrexate area under the curve is an important outcome predictor in patients with primary CNS lymphoma: a pharmacokinetic-pharmacodynamic analysis from the IELSG no. 20 trial. *British Journal of Cancer*. 2010; 102:673–677. [PubMed: 20125159]
11. Ackland SP, Ratain MJ, Vogelzang NJ, Choi KE, Ruane M, Sinkule JA. Pharmacokinetics and pharmacodynamics of long-term continuous-infusion doxorubicin. *Clinical Pharmacology and Therapeutics*. 1989; 45:340–347. [PubMed: 2702792]
12. Piscitelli SC, Rodvold KA, Rushing DA, Tewksbury DA. Pharmacokinetics and pharmacodynamics of doxorubicin in patients with small cell lung cancer. *Clinical Pharmacology and Therapeutics*. 1993; 53:555–561. [PubMed: 8387903]
13. Gamelin E, Delva R, Jacob J, Merrouche Y, Raoul JL, Pezet D, Dorval E, Piot G, Morel A, Boisdron-Celle M. Individual fluorouracil dose adjustment based on pharmacokinetic follow-up compared with conventional dosage: results of a multicenter randomized trial of patients with metastatic colorectal cancer. *Journal of Clinical Oncology*. 2008; 26:2099–2105. [PubMed: 18445839]
14. Engels FK, Loos WJ, van der Bol JM, de Bruijn P, Mathijssen RHJ, Verweij J, Mathot RAA. Therapeutic drug monitoring for the individualization of docetaxel dosing: a randomized pharmacokinetic study. *Clinical Cancer Research*. 2011; 17:353–362. [PubMed: 21224369]

15. Gustafson DL, Rastatter JC, Colombo T, Long ME. Doxorubicin pharmacokinetics: macromolecule binding, metabolism, and excretion in the context of a physiologic model. *Journal of Pharmaceutical Sciences*. 2002; 91:1488–1501. [PubMed: 12115848]
16. Selting KA, Ogilvie GK, Gustafson DL, Long ME, Lana SE, Walton JA, Hansen RA, Turner AS, Laible I, Fettman MJ. Evaluation of the effects of dietary n-3 fatty acid supplementation on the pharmacokinetics of doxorubicin in dogs with lymphoma. *American Journal of Veterinary Research*. 2006; 67:145–151. [PubMed: 16426224]
17. Loveless H, Arena E, Felsted RL, Bachur NR. Comparative mammalian metabolism of adriamycin and daunorubicin. *Cancer Research*. 1978; 38:593–598. [PubMed: 626965]
18. Galpin AJ, Evans WE. Therapeutic drug monitoring in cancer management. *Clinical Chemistry*. 1993; 39:2419–2430. [PubMed: 8222253]
19. Hon YY, Evans WE. Making TDM work to optimize cancer chemotherapy: a multidisciplinary team approach. *Clinical Chemistry*. 1998; 44:388–400. [PubMed: 9474050]
20. Vaughan A, Johnson JL, Williams LE. Impact of chemotherapeutic dose intensity and hematologic toxicity on first remission duration in dogs with lymphoma treated with a chemoradiotherapy protocol. *Journal of Veterinary Internal Medicine*. 2007; 21:1332–1339. [PubMed: 18196744]
21. Frimberger AE, Moore AS, Rassnick KM, Cotter SM, O’Sullivan JL, Quesenberry PJ. A combination chemotherapy protocol with dose intensification and autologous bone marrow transplant (VELCAP-HDC) for canine lymphoma. *Journal of Veterinary Internal Medicine*. 2006; 20:355–364. [PubMed: 16594594]
22. Sorenmo K, Overley B, Krick E, Ferrara T, LaBlanc A, Shofer F. Outcome and toxicity associated with a dose-intensified, maintenance-free CHOP-based chemotherapy protocol in canine lymphoma: 130 cases. *Veterinary and Comparative Oncology*. 2010; 8:196–208. [PubMed: 20691027]

Figure 1.

Scatterplot for 27 dogs evaluating the correlation between the measured DOX exposure (AUC_{0-6h}) using non-compartmental analysis and the predicted exposure using the limited-sampling model consisting of only the 5, 45 and 60 min post-end-of-infusion serum DOX concentrations. The data are highly correlated, suggesting that evaluation of these three serum concentration timepoints can accurately predict DOX exposure.

Figure 2.

Correlation of measured versus predicted DOX exposure for three independent, randomly generated training sets of 15 dogs with the remaining 12 dogs used for validation of each set using the limited-sampling model consisting of 5, 45 and 60 min post-end-of-infusion serum concentrations.

Table 1

Evaluation of DOX serum concentration timepoints as predictors of total exposure

No. of predictors	Correlation range	Best predictor set
1	25.6–70.6	30 min
2	39.4–88.3	5 and 60 min
3	43.8–91.9	5, 45 and 60 min

Table 2Ability of independent training sets to predict exposure ($AUC_{0 \rightarrow 6 \text{ h}}$) of test sets

Parameter	Set 1	Set 2	Set 3
Prediction error	19.1 (11.0–27.0)	3.3 (0–8.2)	5.9 (1.4–10.4)
Absolute prediction error	19.2 (11.3–27.1)	7.1 (4.2–10)	7.6 (4.3–10.9)
Accuracy	84.9 (79.4–90.4)	93.1 (90.4–95.8)	93.4 (90.9–95.9)

Values represent the mean and (95% confidence interval) for the 12 test samples that were not included in the training sets.