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Potency and Stability of Compounded Formulations of Chlorambucil, Melphalan and Cyclophosphamide

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Abstract

Oral chemotherapy agents are frequently compounded in veterinary medicine however, the potency of some formulations have been shown to vary from that of Food and Drug Administration (FDA)-approved products. The objective of this study was to evaluate the potency and stability of three compounded oral chemotherapeutics commonly prescribed to be administered over time. Compounded chlorambucil 1 mg, cyclophosphamide 5 mg and melphalan 1 mg were obtained and tested upon receipt and 6 weeks later. Potency ranged from 71 to 104% for chlorambucil and 58 to 109% for melphalan; 1/4 and 2/4 samples were <90% of labelled strength at baseline and 6 weeks, respectively, for both drugs. Potency of cyclophosphamide ranged from 92 to 107% with all samples +/−10% of labelled strength at all time points. These results demonstrate variability of compounded chemotherapy products, and highlight the need to consider both potency and stability when prescribing orally compounded chemotherapy.

Keywords

Veterinary; chemotherapy; feline; canine; oncology

Introduction

Chemotherapy drugs are commonly compounded in veterinary medicine to ensure smaller pets are safely dosed, to provide drugs in formulations that clients are able to administer to their pet, or when Food and Drug Administration (FDA)-approved formulations are unavailable. However, there is growing concern as to whether compounded medications are similar in efficacy to and of similar quality as FDA-approved products. Previous studies have evaluated omeprazole for horses and itraconazole, trilostane and ciclosporin in dogs and found that these compounded products were not equivalent in potency and resulted in treatment failures more frequently than the FDA-approved versions of the drugs.[1–4]

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Conflict of Interest

The authors do not have any financial or personal relationships to disclose that could inappropriately influence the content of this paper.

Recently, our group demonstrated neutropenia that occurred with greater frequency and severity in dogs treated with FDA-approved formulations of lomustine as compared with dogs administered a compounded product.[5] Additionally, potency of the compounded formulations for 5 different compounding pharmacies ranged from 50–115% of the labelled concentration with only one of the samples within 10% of labelled concentration, which is the requirement for FDA-approved formulations. Evaluation of potential differences between compounded and FDA-approved formulations of lomustine was initiated as there was a clinical suspicion that neutropenia, and possibly tumor response, was occurring less frequently when dogs were treated with compounded lomustine. Potential differences in potency between compounded and FDA-approved product may not be as readily detected clinically with chemotherapy agents that are generally well-tolerated by most dogs and cats, such as chlorambucil, melphalan and metronomic (low-dose) cyclophosphamide. Additionally, these drugs are frequently used to treat more slowly progressing cancers and tend to be administered for a number of weeks before response to treatment can be assessed.[6–9] Side effects associated with cyclophosphamide, melphalan and metronomic cyclophosphamide occur less frequently and predictably than for other cytotoxic drugs and when side effects do occur, it may take weeks to months before they are detected.[8, 10, 11] This raises concerns that recognition of sub-therapeutic dosing could be delayed, resulting in administration of an ineffective cancer therapeutic an extended period of time.

Despite wide use clinically, the potency and stability of compounded oral chemotherapy drugs have not been extensively evaluated to date. A recent publication demonstrated that 40% of samples of compounded cyclophosphamide had an actual potency that was >±10% the labelled concentration of drug; one sample had a potency of <10% of labelled concentration 60 days after receipt, indicating that stability of compounded chemotherapy drugs may be an issue as well.[12] This study raises further concern that compounded chemotherapy agents may not be as stable or as potent as their FDA-approved product. Concerns regarding stability may be particularly critical for drugs that are to be administered over weeks or months. The objective of this study was to further assess variability in potency and stability of compounded formulations of three commonly compounded oral chemotherapy drugs, chlorambucil, melphalan and cyclophosphamide.

Materials and Methods

Compounded oral chemotherapy drugs were purchased from six compounding pharmacies selected based on their advertisement in national veterinary publications and ability to compound chlorambucil, melphalan and/or cyclophosphamide; not all agents were available from all of the pharmacies. Compounded formulations of chlorambucil 1mg, melphalan 1mg and cyclophosphamide 5mg were purchased and stored either at room temperature (RT) or 4C upon receipt and for the duration of the study, as instructed by each compounding pharmacy. If no storage recommendation was made by the supplier, the samples were stored at RT. All samples for each drug were ordered on the same day so that they would be received in a similar time frame and could be analyzed on the same day. Certificate of analyses were not requested from any of the compounding pharmacies, nor did any of the pharmacies provide them on a voluntary basis. Baseline testing occurred within 14 days of initial receipt of the products. The 6-week stability time point was selected as it is common

practice for prescriptions for these chemotherapeutics to be dispensed at our institution in a quantity sufficient to last for 30 to 60 days. The FDA-approved products, chlorambucil 2 mg, melphalan 2 mg and cyclophosphamide 25 mg were obtained from the UC Davis Veterinary Medical Teaching Hospital pharmacy and tested with the compounded products to serve as controls. FDA-approved products were stored as recommended by the supplier: 4C for chlorambucil and melphalan and RT for cyclophosphamide.

The analytical reference standards for chlorambucil, melphalan, and cyclophosphamide were obtained (Sigma Aldrich, St Louis, MO) and each prepared at concentrations of 1 mg/mL of free base. For analysis, working solutions were prepared by dilution of the 1 mg/mL stock solutions with methanol to concentrations of 10 and 100 ng/μL. Calibrators were prepared by dilution of the 10 ng/μL working standard solutions with methanol to concentrations ranging from 1.4 to 2.6 ng/μL for chlorambucil and melphalan, and 0.4 to 1.6 ng/μL for cyclophosphamide.

Prior to analysis the compounded and FDA-approved chlorambucil, melphalan, and cyclophosphamide were dissolved in methanol, methanol:ethanol (1:1 v:v), and methanol:water (1:1 v:v), respectively. Low heat, stirring and crushing was used to aid in dissolution. Samples were brought to volume in volumetric flasks and then serially diluted into the range of the calibration curves. For chlorambucil and cyclophosphamide two pills were extracted for the initial determination. This was repeated at 6 weeks. For melphalan two out of five pharmacies had five pills extracted initially and the other three pharmacies had two pills extracted. Additional sampling of melphalan from the two pharmacies was performed due to discordance of the drug concentration from the first two capsules sampled (Table 1). After six weeks, two pills of each were extracted. All pill extracts were injected three times each into the mass spectrometer. All pills were stored as directed by the pharmacy directions throughout the duration of the study.

The concentrations of the analytes were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS). Quantitative analysis was performed on a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA) coupled with a turbulent flow chromatography system (TFC TLX4, Thermo Scientific, San Jose, CA) having 1100 series liquid chromatography system (Aligent Technologies, Palo Alto, CA) and operated in laminar flow mode. The system was operated using positive heated electrospray ionization). The spray voltage was set at 3500 V, sheath gas and auxillary gas were 40 and 30 respectively (arbitrary units), and the vaporizer temperature was 350°C. Product masses and collision energies were optimized by infusing the standards into the mass spectrometer. Chromatography employed an ACE 3 C18 10cm x 2.1mm column (Mac-Mod Analytical, Chadds Ford, PA) and a linear gradient of acetonitrile (ACN) in water, both with 0.2% formic acid, at a flow rate of 0.4 ml/min. The ACN concentration was held at 5% for 0.33 minutes, ramped to 95% over 6 minutes before re-equilibration at initial conditions.

Detection and quantification was conducted using selective reaction monitoring of initial precursor ion for chlorambucil (mass to charge ratio *(m/z)* 304.1), cyclophosphamide (*(m/z)* 261.0) and melphalan (*(m/z)* 305.0). The response for the product ions for chlorambucil (*m/z* 118.1, 168.1, 192.1, 241.1), cyclophosphamide (*m/z* 63.1, 140.0, 142.1, 233.0) and

melphalan (*m/z* 119.1, 168.1, 246.1, 288.1) were plotted and peaks at the proper retention time integrated using Quanbrowser software (Thermo Scientific, San Jose, CA). Quanbrowser software was used to generate calibration curves and quantitate all analytes by linear regression analysis. A weighting factor of 1/X was used for all calibration curves. The responses were linear and gave correlation coefficients (R^2) of 0.99 or better.

Samples with a potency of ±10% of labelled strength were considered to have acceptable potency.

Results

Compounded chlorambucil 1 mg was obtained from 4 different compounding pharmacies. Two pharmacies indicated that the product should be stored at 4°C, one indicated the product should be stored at RT and the 4th did not specify storage conditions therefore the product was kept at RT. Compounded melphalan 1 mg was obtained from 4 compounding pharmacies. Two pharmacies indicated that the product should be stored at 4°C, one stated to store at RT and one did not provide storage instructions and was also stored at RT. Compounded cyclophosphamide 5 mg was obtained from 5 compounding pharmacies. None of the compounding pharmacies provide storage recommendations for cyclophosphamide and all samples were stored at RT. Beyond use dates (BUD) were provided on the label for all samples obtained; none of the six-week stability testing occurred after the BUD.

Mean potency for the individual samples tested for each chemotherapy drug are provided in Table 1. Potency of compounded chlorambucil ranged from 71 to 104% on day 0 and 73 to 103% on 6 weeks after initial testing (Figure 1A). One out of 4 samples was below 90% potency on both day 0 and 42. Another compounded chlorambucil sample was of adequate potency on day 0 but potency of this product decreased to 74% of the labelled concentration at day 42. The other two samples remained within ±10% of labelled concentration at both day 0 and day 42. Samples 2A and 4A were stored at RT; samples 3A and 5A were stored at 4C (Figure 1A).

Potency of compounded melphalan ranged from 65 to 109% on day 0 and 58 to 109% 6 weeks after initial testing (Figure 1b). One out of 4 samples was below 90% potency on both day 0 and 42. Another compounded melphalan sample was of adequate potency (96%) on day 0 but potency decreased to 89% of the labelled concentration at day 42. The other two samples remained within ±10% of labelled concentration at both day 0 and day 42. Samples 2B and 3B were stored at RT; samples 4B and 5B were stored at 4C (Figure 1B). All compounded melphalan samples decreased in potency over the 6-week study period (range: −6.25 to −12.8%).

Potency of compounded cyclophosphamide ranged from 92 to 107% on day 0 and 90 to 99% 6 weeks after initial testing (Figure 1c). All compounded cyclophosphamide samples remained within ±10% of labelled concentration at both day 0 and day 42; all were stored at RT for the duration of the study. Four out of the five compounded cyclophosphamide samples decreased in potency over the 6-week study period (range: −1.0 to −12.6%).

Discussion

Compounding of pharmaceuticals is essential in veterinary medicine to ensure patients can be treated safely and accurately. This is particularly critical for drugs that have a narrow therapeutic window with the potential for serious significant side effects with over-dosing or therapeutic failure if under-dosing occurs. Our previous research indicated that potency of compounded lomustine was highly variable and as a result, dogs that were treated with compounded lomustine did not develop neutropenia as frequently or to the degree of severity as dogs treated with FDA-approved formulations of lomustine. The oral chemotherapy drugs evaluated for potency in this study do not have as predictable side effect profile as lomustine and when side effects occur, it often occurs after prolonged administration of the drug. Additionally, compounded lomustine is generally administered at the time it is prescribed, whereas many other drugs will be sent home to be administered continuously on a daily or every other day basis, making stability of these compounded product as important as potency. Chlorambucil, melphalan and metronomic (or low-dose) cyclophosphamide are frequently used to treat cancers that respond to or progress after treatment more gradually, such as small cell GI lymphoma in cats, multiple myeloma and incompletely resected soft tissue sarcomas in dogs.[7, 8, 10] As the drugs are generally well-tolerated by dogs and cats and it may take weeks to months before toxicities and/or responses to treatment to develop, it is critical that veterinarians have assurance that the products they are prescribing are as potent and stable as FDA-approved chemotherapy agents to ensure patients that “fail” treatment do so because their cancer did not respond to the drug, rather than receiving sub-therapeutic treatment for prolonged intervals.

As with our previous investigation into the potency of compounded lomustine, we have again elected not to identify the compounding pharmacies from which compounded chlorambucil, melphalan and cyclophosphamide were obtained for analysis. Results of the previous studies evaluating potency of lomustine and cyclophosphamide support that potency of the same drug obtained from the same compounded pharmacy is likely variable over time. As we only obtained samples from compounded pharmacies at one-time point, it would be irresponsible to identify those that had acceptable or substandard product at that single time point. Compounding of chemotherapy drugs is essential in our practice of veterinary oncology and our intent is not to vilify the practice of compounding or any specific compounding pharmacy. Rather, we sought only to investigate the potential inconsistencies that may occur with compounded formulations of chemotherapy.

The variability in potency and stability of compounded drugs could potentially arise from a number of factors including the drugs being compounded, whether the compounding is performed from the FDA-approved product or a bulk substance, the compounding technique and storage duration and conditions. A previous study evaluating the potency and stability of compounded cyclophosphamide demonstrated that 4/10 samples of compounded cyclophosphamide evaluated were <90% of labelled concentration at initial testing, with a fifth sample with a potency of <90% of labelled concentration after a 60 day stability period.[12] All samples of compounded cyclophosphamide obtained in this study were within 90–110% of labeled concentration both at baseline and at the 6-week time point, however, three samples had decreases in potency of 6.2%, 7.5% and 12.6% over the six week period.

This raises the concern that perhaps some of these samples would no longer be of acceptable potency if re-tested at 60 days.

Melphalan and chlorambucil showed a bit more variability in potency than compounded cyclophosphamide in the current study. Storage conditions for FDA-approved melphalan and chlorambucil is 4C, but only two out of four of the compounding pharmacies recommended refrigerated storage for compounded melphalan and chlorambucil. This may have impacted potency for these two drugs to some degree, particularly for chlorambucil sample A4 (Fig 1) which was stored at RT and had the greatest decrease in potency. Storage conditions during the six-week stability period do not fully explain the variability in potency at baseline and at six-weeks as some samples with unacceptable potency at baseline were stored at the proper storage conditions during the course of the study. To better assess the impact of storage temperature on product stability, future studies could be designed to divide compounded samples and controls upon receipt with a portion held at RT and the rest at 4C for the duration of the stability period. This would allow for direct comparisons of differences in potency that may occur for products held at 4C versus RT. Thorough assessment of why such variability may occur and the repeatability of these findings over a period of time was beyond the scope of this work as many of these variables likely occur at the compounding pharmacies prior to dispensing of the compounded product.

Beyond the issue of potency and stability of compounded drugs, prescribers of these medications need to consider the issue of bioequivalence of compounded products as compared to FDA-approved products. The process of and products used for compounding could alter the pharmacokinetic parameters when these oral medications are administered to veterinary patients, however, bioequivalence testing of compounded products has not routinely been performed to date in veterinary medicine. However, compounded omeprazole administered to horses and compounded intraconazole administered to healthy research dogs have previously demonstrated that substantial pharmacokinetic alterations may occur when compared to the FDA-approved products.[3,4] Further work is need to assess bioequivalence of compounded products in clinical veterinary patients to better understand the potential therapeutic impact beyond potency testing alone.

In conclusion, these findings, in combination with previous investigations into the variability of potency of compounded drugs, should stimulate veterinarians to carefully weigh the benefits and potential risks of prescribing compounded formulations of chemotherapy drugs. The observed decreases in potency of some compounds over time may also discourage prescribing or ordering large quantities of compounded drugs that are to be continuously administered, particularly in light of the decreasing potency of some of these compounded products despite testing well within the BUD provided by the compounding pharmacy. Lastly, if greater oversight or voluntary assurance testing of compounds by individual compounding pharmacies are instituted in the future, both potency and stability should be considered.

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Abbreviations

ACN	acetonitrile
BUD	beyond use date
FDA	Food and Drug Administration
LC-MS/MS	liquid chromatography tandem mass spectrometry
m/z	mass to charge ratio
RT	room temperature
TFC	turbulent flow chromatography system
UV	ultraviolet

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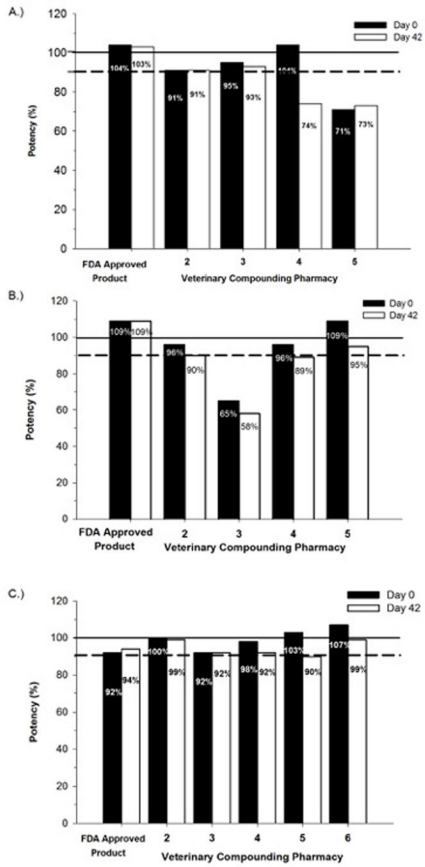

Figure 1.

Bar graphs of the percent potency of compounded A) chlorambucil with a labelled concentration of 1 mg, B) melphalan with a labelled concentration of 1mg, and C) cyclophosphamide with a labelled concentration of 5mg. The solid line indicates 100% of labeled concentration and the dashed line represents 90% of labeled concentration, which is the minimum potency allowed for FDA-approved products. Compounding pharmacies labelled 2 through 5 (or 6) in each figure do not represent the same pharmacy in A, B and C. FDA-approved product was used as a control in each experiment: A) chlorambucil 2mg, B) melphalan 2 mg, and C) cyclophosphamide 25 mg.

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Table 1

Mean potency (±SD) testing of compounded and FDA-approved chlorambucil, melphalan and cyclophosphamide

	Chlorambucil		Melphalan		Cyclophosphamide	
	Day 0	**Day 42**	**Day 0**	**Day 42**	**Day 0**	**Day 42**
FDA Approved Product						
Rep 1	104 ± 3%	108 ± 17%	106 ± 1%	109 ± 1%	89 ± 1%	91 ± 1%
Rep 2	104 ± 1%	97 ± 16%	111 ± 3%	109 ± 1%	95 ± 0%	97 ± 0%
Pharmacy 2						
Rep 1	91 ± 1%	84 ± 6%	138 ± 2%	91 ± 1%	102 ± 2%	99 ± 1%
Rep 2	91 ± 2%	98 ± 11%	79 ± 7%	89 ± 8%	98 ± 0%	99 ± 0%
Rep 3			91 ± 2%			
Rep 4			79 ± 6%			
Rep 5			94 ± 6%			
Pharmacy 3						
Rep 1	95 ± 2%	78 ± 2%	83 ± 1%	65 ± 0%	94 ± 1%	92 ± 0%
Rep 2	95 ± 0%	108 ± 1%	55 ± 4%	50 ± 0%	90 ± 0%	92 ± 1%
Rep 3			58 ± 2%			
Rep 4			56 ± 2%			
Rep 5			71 ± 10%			
Pharmacy 4						
Rep 1	102 ± 2%	74 ± 2%	91 ± 2%	90 ± 1%	98 ± 1%	93 ± 1%
Rep 2	105 ±1%	74 ± 4%	100 ± 7%	88 ±1%	98 ± 0%	90 ± 0%
Pharmacy 5						
Rep 1	70 ± 2%	52 ± 0%	94 ± 2%	94 ± 1%	100 ± 1%	90 ± 6%
Rep 2	71 ± 1%	94 ± 1%	124 ± 3%	96 ± 9%	106 ± 3%	79 ± 2%
Pharmacy 6						
Rep 1					107 ± 1%	99 ± 1%
Rep 2					107 ± 0%	99 ± 1%

SD, standard deviation