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Dietary composition alters methotrexate toxicity without changing its pharmacokinetic parameters in cats

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This study determined the effect of a commercial dry expanded (complex) diet and purified diet on methotrexate (MTX) toxicity and pharmacokinetics in the cat. Twelve cats were randomized to receive a purified diet or a complex diet for 21 days. They were then given an intravenous bolus injection of MTX at a dose of 10 mg/kg (160 mg/M^2). Cats fed the purified diet had severe enteritis, characterized by depression, lethargy, diarrhea, and vomiting. Cats receiving the complex diet remained mentally bright, alert, and responsive throughout the post-chemotherapy period, and only one of six cats in this dietary group developed mild diarrhea 48 hr after MTX administration. Methotrexate was associated with a significant ($P < 0.0001$) reduction in the total white blood cell (WBC) counts in cats receiving the purified and complex diets.

The elimination of MTX appeared to follow a three-compartment model of elimination. Plasma MTX concentrations at all time points examined were virtually identical in cats receiving the purified and complex diets. The terminal half-life of MTX in cats receiving the complex and purified diets was 1.75 ± 0.44 hr and 2.77 ± 0.8 hr, respectively. The similarity of plasma MTX concentrations and pharmacokinetic parameters in both dietary groups suggests that dietary alterations may influence enterotoxicity directly rather than altering exposure to MTX. (J. Nutr. Biochem. 8:79–84, 1997.) © *Elsevier Science Inc. 1997*

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Introduction

Methotrexate (MTX) is a potent inhibitor of the enzyme dihydrofolate reductase and has been shown to be effective in the treatment of various malignancies in human and animal patients.[1,2,3] A major factor limiting the use of MTX is its toxicity toward nonmalignant, rapidly proliferating tissue, especially that of the small intestine and bone marrow.[4] The gastrointestinal toxicity of MTX has been demonstrated to be influenced by the type of diet the animal is consuming.[5–13] The administration of MTX to rats maintained on elemental diets fed ad libitum results in severe enterotoxicity and death, whereas MTX-induced enterotoxicity is prevented when rats are maintained on a dry expanded (complex) diet.[7–13] The increased toxicity observed in rats receiving the elemental diets may have been associated with malnourishment after MTX administration. Well-nourished cancer patients are more tolerant to chemotherapy than malnourished patients and nutritional repletion in experimental animals is associated with reduced host toxicity to MTX.[14,15] No studies have compared the toxicity and pharmacokinetic alterations associated with MTX administration to animals fed purified or complex diets that meet the animals daily caloric requirements. The identification of dietary components that alter chemotherapy-induced enterotoxicity is of fundamental importance to maximize the antineoplastic activity of the drug while minimizing intestinal toxicity.

MTX is an antimetabolite that is included in multidrug protocols at veterinary institutions for therapy of lymphoreticular neoplasms and myeloproliferative disorders in

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cats.[3] MTX was chosen for study due to its enterotoxic properties and because plasma drug concentrations in experimental animals have been shown to correlate with host toxicity. Specifically, increased plasma MTX concentration over time directly relates to the occurrence and degree of adverse drug effects, whereas the magnitude of peak plasma drug concentration bears little relationship to clinical toxicity.[16]

We have developed a feline model of MTX-induced enteritis to evaluate the influence of qualitative alterations of dietary intake on intestinal mucosal integrity. The model was developed in a phase 1 study using a dose escalation regime (modified Fibonacci series[17]) of MTX that ultimately resulted in reduced food intake, diarrhea, and vomiting without causing any undue morbidity or mortality in cats. MTX was administered as an intravenous bolus injection starting at a dose of 0.8 mg/kg that has been recommended for cats with lymphoma.[3] The Fibonacci series uses a dose escalation regime whereby the second step is twice the starting dose, the third step is 67% greater than the second, the fourth step is 50% greater than the third, the fifth step is 40% greater than the fourth, and each subsequent step is 33% greater than that preceding it.[17] The MTX dose of 10 mg/kg was associated with enterotoxicity manifested by reduced food intake, diarrhea, and vomiting in cats receiving a dry expanded (complex) diet. In addition, small intestinal biopsies from these cats were characterized by villous blunting and crypt necrosis.

MTX is directly toxic to the intestinal mucosa and is eliminated primarily in the urine.[18] One mechanism for the enhanced toxicity seen in rats fed a purified diet could be an alteration in drug pharmacokinetics, because increased plasma levels of MTX over time correlates directly with increased toxicity.[19] We hypothesized that administration of a purified diet to cats would delay the clearance of MTX resulting in enhanced toxicity to the drug.

The objectives of the present study were 2 fold: 1) to determine whether MTX enterotoxicity in cats could be ameliorated by feeding a dry expanded (complex) diet, and 2) to examine the pharmacokinetic alterations in MTX metabolism occurring after administration of purified and complex diets.

Methods and materials

Animals

Twelve adult specific pathogen-free cats (eight male and four female) weighing 2.5 to 5.4 kg (mean ± SEM: 3.9 ± 0.3 kg) were obtained from the Nutrition and Pet Care Center, University of California at Davis. The cats were housed in individual metabolism cages and underwent an acclimatization period of 7 days in a constant temperature environment with 12-hr light/dark cycle. During this period they received a commercial dry expanded (complex) diet that was meal-fed twice daily for a period of 2 hr, and the cats had free access to water at all times. Cats were maintained according to the Guide for the Care and Use of Laboratory Animals (NRC 1985) and the Animal Welfare Act. The protocol was approved by the Committee for Care and Use of Laboratory Animals, University of California, Davis.

Diets

Two diets were used in the experiment. The complex diet (*Table 1*A and B) consisted of intact protein sources (ground yellow corn, chicken byproduct meal, soybean meal, ground wheat, digest of poultry byproducts, meat, dried milk protein, tuna meal) and had a similar proximate analysis on a dry matter basis (crude protein 33.7%, fat 10.3%, crude fiber 2.1%, ash 7.6%) to standard commercial dry expanded diets formulated for the maintenance of adult cats. The purified diet (*Table 1*A and B) was prepared in our laboratory and contained starch, dextrose, crystalline amino acids (30% protein), vegetable oil, animal tallow, and trace elements and vitamins. The dietary concentration of protein, vitamins, and minerals in both diets met or exceeded the National Research Council (1986) requirements.

Design

Twelve cats were randomized to receive either the purified diet or the complex diet after the 7-day adaptation period. Cats were placed under general anesthesia on day 0 to obtain baseline endoscopic biopsies of the stomach, duodenum, and colon. Percutaneous endoscopic gastrostomy (PEG) tubes (Mill-Rose Pezzer PEG catheter, Mill-Rose Laboratory Inc., Mentor, OH USA) were placed to facilitate the isocaloric feeding of the purified and complex diets. All cats were fed their respective diets twice daily to meet their daily caloric requirement 1.4[(BW (kg) × 30) + 70] kcal/day.[20] Experimental diets were meal fed twice daily for 21 days before MTX sodium (Lederle Laboratories Division, American Cyanamid Company, Pearl River, NY USA) was injected via cephalic vein at the predetermined dosage of 10 mg/kg body weight.

Pharmacokinetic analysis

All MTX injections were performed approximately 2 hr after the morning feeding to eliminate any variation between the two experimental groups due to diurnal variation in drug toxicity. Serial heparinized blood samples (approximately 2 mL each) were obtained before and for up to 64 hr post-MTX administration by venipuncture of the jugular vein. All blood samples were immediately centrifuged and subsequently frozen at −70°C until analyzed. Both groups of cats were maintained on their prescribed diets for 3 days after the administration of MTX, and the cats were observed twice daily for evidence of toxicity. MTX concentration in the plasma was measured by a quantitative fluorescence polarization immunoassay on a TDx System (Abbott Laboratories, Ab-

Table 1A Essential amino acid composition (g/kg) of the purified and complex diets

Amino acid	Purified diet	Complex diet
Arginine	22.5	22.0
Histidine	6.8	9.0
Isoleucine	11.2	12.0
Leucine	27.0	25.0
Lysine	22.5	19.0
Methionine	8.9	6.0
Cystine	7.9	4.0
Phenylalanine	8.9	14.0
Tyrosine	7.9	9.0
Threonine	13.6	14.0
Tryptophan	3.4	2.0
Valine	13.6	15.0
Taurine	1.5	1.2

Table 1B Dispensable amino acid composition (g/kg) of the purified and complex diets

Amino acid	Purified diet	Complex diet
Alanine	50.0	16.0
Glycine	40.0	22.0
Asparagine	30.0	*
Proline	20.0	20.0
Glutamine	0	*
Aspartate	0	17.0
Serine	0	13.0
Glutamate	0	13.0

*Glutamine and asparagine content of the complex diet are unlisted, but were calculated to be approximately 35g/kg and 30 g/kg, respectively.

bott Park, IL, USA). The lower limit of sensitivity of the method is 0.05 μmol/L, and the intraassay and interassay coefficient of variation for the assay is between 4 and 8% and 5 and 9%, respectively. Data on the plasma concentrations of MTX from individual cats from each treatment group ($n = 6$) were analyzed using Model 18 of the WINONLIN library (Statistical Consultants, Inc., Lexington, KY USA). This program used a three-compartment model with a bolus intravenous input into the central compartment and first order elimination from the central compartment. Non-linear least squares regression with Gauss-Newton minimization and uniform weighting was employed to estimate the reported pharmacokinetic parameters.

Evaluation of toxicity

A comprehensive performance chart was recorded twice daily for all cats and contained observations pertaining to mental status and adverse effects to MTX (vomiting, diarrhea, lethargy, retching, salivation). All cats had a minimum data base (complete blood count, serum biochemical profile, and urinalysis) performed on days 0, 21, and 24 of the study.

Statistical analysis

All values are expressed as the mean ± standard error (SEM). Repeated measures analysis of variance was used to compare diet groups and measurements taken either before and after treatment (hematologic parameters) or sequentially across time on the same individual (MTX concentrations). In the latter case orthogonal decomposition of trend was used to characterize the rate of change across time. Interactions between diet group and dependent measures were also evaluated. A type-I error rate of less than 5% was considered statistically significant.

Results

Clinical performance status

Diarrhea was not observed in any cats during the 7-day acclimitization period. Forty-eight hr after MTX administration, lethargy, depression, and diarrhea were noted in 4/6 cats receiving the purified diet. The severity of diarrhea increased among these four cats over the ensuing 24 hr. One of six cats in the complex diet group developed mild diarrhea 48 hr after MTX administration; however, all cats in the complex diet group remained mentally bright, alert, and responsive throughout the post-chemotherapy period. Vomiting was not observed in any cats receiving the complex diet pre- or post-MTX administration; however, vomiting was noted in two cats receiving the purified diet before MTX was administered and in two cats after MTX injection.

Hematologic findings

Segmented neutrophils, lymphocytes, monocytes, and eosinophils were significantly decreased by MTX administration in both dietary groups ($P < 0.0001$, $P = 0.0001$, $P = 0.014$, and $P = 0.036$, respectively) (*Table 2*). The average total WBC count in cats receiving the complex diet decreased from 16083 ± 1021/μL to 11783 ± 1344/μL 3 days after MTX administration. In contrast, the total WBC count in cats receiving the purified diet decreased from 21150 ± 1650/μL to 10067 ± 1455/μL 3 days after MTX administration. The hematocrit in cats receiving the complex diet significantly decreased ($P = 0.0001$) from 39.5 ± 2.4% to 33.2 ± 2.2% 3 days after MTX administration. The hematocrit in cats receiving the purified diet significantly decreased ($P < 0.0001$) from 39.8 ± 1.6% to 31.3 ± 1.02% 3 days after MTX administration. No abnormalities were detected on the serum biochemical profiles and urinalysis of cats on complex or purified diets before or after MTX administration.

Pharmacokinetic findings

The plasma concentrations of MTX (μmol/L) at various times after the intravenous injection of the drug at a dose of

Table 2 Hematologic results in cats receiving complex or purified diets pre- and post-methotrexate administration

	Complex pre-MTX	Complex post-MTX	Purified pre-MTX	Purified post-MTX	P*
PCV	39.5 ± 2.4	33.2 ± 2.2	39.8 ± 1.6	31.3 ± 1.0	$P < 0.0004$
Hb	12.3 ± 0.7	10.2 ± 0.7	12.5 ± 0.6	10.0 ± 0.4	$P > 0.05$
MCV	45.5 ± 0.3	46.2 ± 5.0	45.2 ± 0.4	45.6 ± 0.6	$P > 0.50$
WBC	16083 ± 1021	11783 ± 1344	21150 ± 1650	10067 ± 1455	$P < 0.0001$**
Neutrophils	9246 ± 939	7626 ± 1109	14304 ± 1120	6963 ± 1376	$P < 0.0001$**
Lymphocytes	5978 ± 578	3693 ± 445	5923 ± 906	2728 ± 359	$P = 0.0001$
Monocytes	280 ± 64	183 ± 50	426 ± 154	125 ± 47	$P = 0.014$
Eosinophils	548 ± 172	281 ± 78	497 ± 134	214 ± 72	$P = 0.036$

*Test of hypothesis that Pre-MTX values = Post-MTX values.
**Differences in the magnitude of the reduction were significantly different between the dietary groups ($P < 0.04$).

10 mg/kg body weight (160 mg/M^2) are presented *in Figure 1*. No significant difference in absolute MTX concentrations or in the rates of decline of MTX concentrations over time was noted between the two dietary groups, which is apparent from the nearly superimposable curves. The pharmacokinetic analysis of MTX was performed on individual cats from both dietary groups. The elimination of MTX from cats when plotted on semilogarithmic graph paper demonstrates curvilinear kinetics. This suggests that kinetic processes other than simple first-order elimination are present. Analysis of this data suggests that MTX in cats appears to follow a three-compartment model of elimination that has been reported previously in humans.[21–23] The pharmacokinetic constants ± the standard deviation (SD) for each treatment group are presented in *Table 3*. Using these parameters it is possible to calculate the plasma concentration of MTX at various times after the administration of the drug. The equation that best describes this relation is $C_t = A*e^{(-\alpha*t)} + B*e^{(-\beta*t)} + C*e^{(-\gamma*t)}$, using the values for each of these variables presented in *Table 3*. The lines represented in *Figure 1* are the calculation of the concentrations using this equation and the reported values for A, B, C, α, β, and γ. The goodness-of-fit of these lines suggests that this is the appropriate method of analysis for this data.

Discussion

The similar plasma concentrations of MTX observed in both dietary groups, despite marked differences in observed toxicity, demonstrate that plasma MTX concentrations were not indicative of toxicity. These results suggest that qualitative dietary differences may have influenced enterotoxicity directly rather than changed the duration of exposure to MTX. These findings agree with those reported by Funk and Baker who observed no differences in MTX pharmacokinetics in rats receiving purified and complex diets, despite significant differences in enterotoxicity.[12] The mechanisms for the differences in enterotoxicity are complex and poorly understood, although the presence of fiber, intact protein sources, glutamine, and nucleosides in the complex diet may have accounted for the intestinal protective effects found in cats receiving this diet.[8,11,12,24] The most likely reason for the vomiting observed in four cats fed purified diets before MTX administration was the overzealous infusion of the hyperosmolar solution intragastrically. This hypothesis is supported by the fact that almost all vomiting episodes occurred during the rapid feeding of the purified diet, and slow refeeding of the diet approximately 20 min after each vomiting episode was not associated with subsequent vomiting in any cats. In addition, the vomiting episodes observed in the two cats fed the purified diet after MTX administration were not associated with feeding.

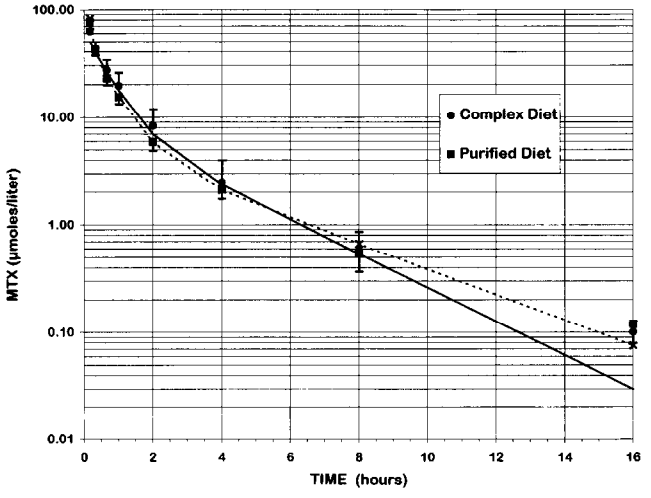

Figure 1. Semilogarithmic plot of mean (± SD) plasma methotrexate (MTX) concentrations (µmol/L) following intravenous administration to cats receiving complex (●) or purified diets (■). The line represents the estimated concentrations using the pharmacokinetic parameters reported in *Table 3* and the equation $Ct = A*e^{(-\alpha*t)} + B*e^{(-\beta*t)} + C*e^{(-\gamma*t)}$. Solid line = complex diet; Dashed line = purified diet.

MTX injection resulted in significantly lower WBC counts in both dietary groups; however, the post-MTX WBC counts obtained 3 days post-MTX injection were well above the low-to-normal value (6000/µL) reported for the University of California, Davis, VMTH, and were probably not reflective of the leukopenic nadir. Leukopenia reaches its nadir 7 to 10 days after MTX administration in humans[25] and after 4 to 6 days in dogs treated with high-dose MTX (3–6 g/M^2).[26] In addition, the greater decrease in the WBC and neutrophil counts observed in the purified diet-fed cats after MTX administration was not clinically meaningful because these cats had higher WBC and neutrophil counts before MTX administration. The greater sensitivity of the intestine versus bone marrow found in this study reflects the lower threshold plasma concentration of MTX required to inhibit DNA synthesis in intestinal epithelium (5×10^{-9} moles/L)[27] and the greater accumulation and persistence of MTX in intestinal epithelium as opposed to bone marrow.[28]

The severity of MTX cytotoxicity is influenced predominantly by the drug concentration and duration of exposure.[29,30] In tissue culture and in intact animals, extracellular drug concentrations of 10^{-8} moles/L are required to inhibit thymidylate synthesis in normal bone marrow and exert an antineoplastic effect in murine tumor cells.[4] In comparison to drug concentration, the duration of exposure to MTX is a more critical factor in determining cell death if the threshold concentration for cytotoxicity is exceeded.[4] Studies in mice have demonstrated that extracellular concentrations of MTX at 5×10^{-8} moles/L for 72 hr produce the same cytotoxicity as MTX concentrations of 1×10^{-5} moles/L for 12 hr.[30] The relationship between cell kill and duration of tissue exposure is probably a reflection of the S-phase specificity of MTX. The other variables that influence the antitumor activity include the amount of MTX actually delivered to the tumor site, the amount of drug taken up by the tumor itself, the extent of polyglutamation within the tumor cell, and the phase of the cell cycle at the time of exposure.[29]

The plasma pharmacokinetics of MTX has been found to be an independent predictor of relapse in children treated during the maintenance phase of acute lymphocytic leukemia.[31–33] The risk of relapse was significantly increased in children with a rapid drug clearance and steady-state MTX concentration of less than 16 µmol/L.[31] All cats in this

Table 3 Pharmacokinetic parameters of methotrexate (MTX) after intravenous administration of 10 mg/kg to cats receiving a complex or purified diet

		Complex		Purified	
	Unit	Mean	S.D.	Mean	S.D.
A	µmol/L	505	382	280	255
B	µmol/L	51.7	11.1	47.1	14.2
C	µmol/L	9.5	6.2	5.8	3.7
α	hr^{-1}	39.6	35.9	11.7	7.7
β	hr^{-1}	1.6	0.6	1.5	0.5
γ	hr^{-1}	0.4	0.2	0.3	0.09
C Max	µmol/L	566	379	333	272
Volume	L/kg	0.03	0.02	0.04	0.02
t½α	hr	0.03	0.02	0.07	0.03
t½β	hr	0.6	0.4	0.5	0.1
t½γ	hr	1.8	0.4	2.8	0.8
Cl	ml/min/kg	1.66	0.0008	1.66	0.000
AUC	µmol.hr/L	84.6	34.1	74.4	11.5
AUMC	µmol.hr/L	89.3	32.3	103.1	24.2
MRT	hr	1.2	0.3	1.4	0.3
Vss	L/kg	0.2	0.05	0.2	0.07

A Zero time intercept associated with the α phase.
B Zero time intercept associated with the β phase.
C Zero time intercept associated with the γ phase.
α Macro rate constant associated with the α phase.
β Macro rate constant associated with the β phase.
γ Terminal elimination rate associated with a three-compartment model.
Cmax Maximum plasma drug concentration.
Vol Apparent volume of distribution.
t½-α Elimination half-life derived by dividing 0.693/α.
t½-β Elimination half-life derived by dividing 0.693/β.
t½-γ Elimination half-life derived by dividing 0.693/γ.
Cl Plasma clearance rate.
AUC Area under the curve.
AUMC Area under the moment curve.
MRT Mean residence time.
Vss Volume of distribution at steady state.

study showed plasma MTX concentrations below this value from as early as 1 hr post-MTX administration, despite the administration of MTX in excess of 12 times the recommended therapeutic dose for cats with lymphoma.[3] Methotrexate plasma concentrations were below the detection limits of the assay at times greater than 16 hours post-MTX injection. The observed terminal half-lives of 1.75 ± 0.44 hr and 2.77 ± 0.8 hr for cats receiving the complex and purified diets, respectively, were significantly shorter than the value of 8 to 10 hr reported for humans.[23,34] The rapid elimination of MTX and low plasma concentrations attained in these cats is consistent with the poor clinical benefit derived in lymphoma patients receiving MTX when it is utilized at the recommended dose of 0.8 mg/kg body weight.[3] It seems logical that plasma therapeutic concentrations could be better maintained with a continuous infusion or with frequent bolus injections in the cat in light of methotrexate's S phase specificity and short half life.

In conclusion, the similarity of plasma MTX concentrations in the purified and complex dietary groups despite marked differences in toxicity suggest that dietary composition may have accounted for the protection from the adverse gastrointestinal clinical signs observed in cats receiving the complex diet.

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