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Reports

The Effect of Commonly Used Vehicles on Canine Hematology and Clinical Chemistry Values

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Drug metabolism and pharmacokinetic (DMPK) studies are an important phase in drug discovery research. Compounds are administered via the intravascular or extravascular routes to animals to calculate various pharmacokinetic parameters. An important step in this process is dissolving the novel compound in a safe vehicle. This procedure is particularly challenging for compounds that must be administered intravenously, as the solution must be clear before injection. There are no published guidelines on which vehicles, or combination of vehicles, are acceptable in a particular species, nor are there published data on the effects these vehicles have on clinical chemistry or hematology parameters, particularly in dogs. In this study, 9 vehicles commonly used at sanofi-aventis USA (propylene glycol, polyethylene glycol 400, glycofurol, hydroxypropyl β-cyclodextrin, dimethyl sulfoxide, N-methyl-2-pyrrolidone, dimethylacetamide, ethyl alcohol, and saline) were tested for adverse clinical reactions (such as vomiting or diarrhea) and for their effect on hematology and clinical chemistry parameters. Each vehicle was administered to a group of 8 Beagles by slow intravenous infusion, and blood was collected prior to infusion and at 24 h and 7 d postinfusion. Of 8 dogs given propylene glycol, 2 developed mild gastrointestinal signs (vomitus, diarrhea) after their infusions. None of the vehicles tested induced significant hematology or serum clinical chemistry abnormalities, nor were significant clinical signs noted after administration. We conclude that at the dose, route, and manner described, all of the vehicles tested in this study are clinically safe to use and have no acute effects on hematology or serum chemistry parameters.

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BCG, bromocresol green; BUN, blood urea nitrogen; CBC, complete blood count; DMA, dimethylacetamide; DMSO, dimethyl sulfoxide; DMPK, drug metabolism and pharmacokinetics; GLDH, glutamate dehydrogenase; LD_{50}, lethal dose 50; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NMP, N-methyl-2-pyrrolidone; PEG, polyethylene glycol; RBC, red blood count

Discovery research in the pharmaceutical industry requires the conduct of metabolism and pharmacokinetic studies to determine the absorption, bioavailability, and excretion of novel compounds. A crucial factor in these studies is optimizing the delivery of test substances to the sites of action. A major challenge is to ensure that all compounds, including those that are poorly water-soluble, can be put into a solution that can be administered intravenously, because even drugs intended for oral use are routinely tested by this route. To this end, scientists use a variety of vehicles in the conduct of drug metabolism and pharmacokinetic (DMPK) studies. The ideal vehicle should readily dissolve all compounds, be nontoxic and nonirritating, cause minimal to no red blood cell hemolysis, and exert no pharmacological activity.[11] Because there is no single 'ideal' vehicle for the wide range of compounds evaluated, selection is often based on personal experience.

Although there are a number of publications concerning the use of nonaqueous solvents as vehicles, most involve rodents, and many of the rodent studies involve determination of the dose lethal to 50% of the population (LD_{50}).[2,6,9-11] Very few vehicles have been examined for their acute affects on dogs. In 2 separate studies, propylene glycol was shown to have no affect on blood pressure, nor was there evidence of cardiotoxicity when administered intravenously to dogs.[1,14] No toxicity was noted after acute or chronic administration of 50, 100, and 400 mg/kg of hydroxypropyl β-cyclodextrin intravenously to dogs.[4,8] In contrast, Losher and colleagues noted significant adverse reactions (facial edema, salivation, lacrimation), as well as behavioral abnormalities (excitation, ataxia, 'wet-dog shakes'), when 65% glycofurol was administered intravenously to dogs.[8] Aside from these few studies, the data are lacking as to the physiologic effect various vehicles may have on dogs. In the present study, the 9 vehicles (8 solvents plus saline) used most often at this institution were administered at the dosages and routes used in our DMPK studies, and we assessed the acute affects of these vehicles on canine hematology and clinical chemistry parameters.

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Materials and Methods

Animals. Sixteen male Beagle dogs (15 from Marshall Farms, North Rose, NY; 1 from Covance, Cumberland, VA) ranging in age from 19 to 36 mo were used for this study. All dogs were vaccinated prior to arrival against common viral (canine distemper virus, canine parvovirus, canine adenovirus 2, parainfluenza virus, and rabies virus) and bacterial (*Leptospira interrogans* and *Bordetella bronchiseptica*) pathogens. In addition, the dogs from Marshall Farms were vaccinated for canine oral papilloma virus. The dogs received yearly physical examinations and vaccinations, as well as dental prophylaxis as needed, but none of the dogs had been used on other experimental protocols. They were

housed in stationary modular stainless-steel pens measuring 36 in. wide × 73 in. deep (91 × 185 cm). The pens have a removable side panel, allowing the dogs to be pair-housed during the day. The dogs were fed approximately 6 to 8 oz of PMI Canine Diet #5L18 (Richmond, IN) once daily and had access to water ad libitum. Environmental conditions included 10 to 15 fresh air changes per hour, a temperature range of 20 to 22 °C, relative humidity between 40% and 70%, and a 12:12-h light:dark cycle. The sanofi-aventis Institutional Animal Care Use Committee approved this study, and the dogs were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals*[13] at a facility whose program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Vehicle preparation. Propylene glycol (USP), methyl sulfoxide (ACS spectrophotometric grade), 1-methyl-2-pyrrolidinone (HPLC grade), N, N-dimethylacetamide (HPLC grade), polyethylene glycol (molecular weight, 400; lot, 072K0245), and 2-hydroxypropyl-β-cyclodextrin (lot 102K1231) were obtained from Sigma-Aldrich (Milwaukee, WI). Tetraglycol (glycofurol, lot 2797F) was obtained from ICN Biomedicals (Aurora, OH) and ethyl alcohol (200 proof, USP) was obtained from AAPER Alcohol and Chemical (Shelbyville, KY). All vehicles were prepared in sterile 5.0% dextrose injection solutions (USP; B Braun Medical, Irvine, CA) as volume/volume solutions at the concentrations given in the text, with the exception of 40% 2-hydroxypropyl-β-cyclodextrin, which was prepared as weight/volume. All vehicle solutions were prepared as a single batch and used on the same day.

Standard clear glass serum vials with their associated septa were submerged in boiling distilled water for at least 5 min prior to filling. The vehicle solutions were sterilized by filtration by using 150-ml 0.22-µm GP Express Plus Stericup filtration units (Millipore, Bedford, MA).

Method. The dogs were randomly divided in 2 groups of 8 dogs. Each group was used to test saline and 4 of the 8 solvent vehicles. In addition to saline, Group A dogs received the following vehicles, in order of administration: 30% propylene glycol, 30% polyethylene glycol 400 (PEG), 50% glycofurol, and 40% hydroxypropyl β-cyclodextrin. Group B dogs also received saline and the following vehicles, in order of administration: 10% dimethyl sulfoxide (DMSO), 50% N-methyl-2-pyrrolidone (NMP), 10% dimethylacetamide (DMA), and 10% ethyl alcohol. The volume administered for each vehicle was 1 ml/kg except for NMP, which was administered at 0.25 ml/kg. NMP was administered at a lower dose than the other solvents because higher doses have been found to be toxic in rodents.[7] The dogs had at least 2 wk between each vehicle administration.

Dogs were fasted for approximately 20 h prior to the preinfusion blood draw. The morning of each vehicle administration, each dog was weighed and body temperature was obtained and recorded. Approximately 8 cc of blood was collected from the jugular vein (cephalic vein used on occasion, if necessary) by using a Vacutainer system (Becton, Dickinson and Company, Preanalytical Solutions, Franklin Lakes, NJ). A behavioral log was maintained for each dog, representing the animal's stress and activity level during bleeding (quiet versus struggling).

After the preinfusion blood sample was collected, a 22-gauge 'over-the-needle' catheter was placed aseptically into the cephalic vein. Catheter sites were alternated between right and left cephalic veins for each infusion to maintain vascular integrity over the course of the study. Vehicle was administered through the intravenous catheter by using a Harvard infusion pump (Harvard Bioscience, Holliston, MA); the rate (ml/min) was calculated to allow administration of the vehicle over 10 min. Once the infusion was completed, the catheter was removed, and the dogs were returned to their home cages and observed periodically throughout the day for any clinical signs. They were fed 3 h after vehicle administration. Additional blood samples were collected 24 h and 7 d postvehicle administration. Hematology samples were processed the day the samples were collected. For serum chemistry testing, blood was centrifuged within 30 min of collection, and the serum stored at –70 °C until all 3 samples (preinfusion, days 1 and 7) could be processed together.

Analysis of samples. Hematology and serum clinical chemistry analyses were performed on-site in the clinical pathology laboratory. Complete blood counts (CBC) were analyzed using an Advia 120 hematology analyzer (Bayer Healthcare, Norwood, MA) that utilizes the laminar flow, sheath/stream theory. Cell-by-cell measurements of light scatter and absorption were obtained using a tungsten-halogen light source. Hemoglobin reagent mixed with the sample aliquot, and the absorption of the reaction was measured at 546 nm. Red blood cell count, hematocrit, mean corpuscular volume, hemoglobin, platelet number, reticulocyte number, total white blood cell count, and differential cell analyses were measured directly on the Advia 120, whereas mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean platelet volume, and reticulocyte percent were manually calculated**.**

The serum chemistry parameters were analyzed using a Hitachi 917 (Roche Diagnostics Corporation, Indianapolis, IN), an automated chemistry analyzer. The samples were quantitatively transferred from the sample disk location into a reaction cell. At specifically timed intervals, 1 or 2 reagents (depending on the assay) were quantitatively added to the reaction cell and mixed. As the cell rotated through the photometer, light path measurements were taken bichromatically, sent to a computer, and converted into units of concentration. Electrolytes (Na^+, K^+, and Cl^-) were measured by potentiometry using ion-selective electrode cartridges (Table 1).

Statistical analysis. Mixed linear models that accounted for the relationship among repeated observations over baseline day 1 and day 7 within each animal were fit to each of the observed blood parameters. This relationship was accommodated using a compound-symmetry covariance structure. Raw *P* values contrasting means resulting in the different analyses were adjusted using a stepped Sidak *P* value multiplicity adjustment.[5]

Three different sets of analyses were performed: a baseline assessment across groups, a group assessment for saline only, and an evaluation of solvents compared with saline. The most complex model required for any single blood parameter was fit to all blood parameters within an analysis set. This approach increased the number of pairwise comparisons performed relative to a procedure that allowed for simplified models for some blood parameters; however, the overall interpretability of the analysis was simplified by using a stepped Sidak multiplicity adjustment. All models were fit using PROC MIXED in SAS v8.02 TS Level 02M0 (SAS, Cary, NC).

***Baseline assessment*.** The mixed model

$$y_{ijk} = \mu + group_i + trt_j + animal_k + e_{ijk}$$

was applied to the baseline data only, where µ is the grand mean and *groups* and *treatments* (*trt*) are the effects of interest. The *animal* identified the experimental unit on which repeated measurements were observed across the different treatments at baseline, and *e* is the random error associated with an observed blood parameter reading. The compound symmetric structure specifies the variance of each observation as

Table 1. Hematologic and serum clinical chemistry variables, units, and methods of measurement

Variable	Units	Method or instrumentation
Hematology		
WBC	$\times 10^3/\mu l$	Advia 120
RBC	$\times 10^6/\mu l$	Advia 120
Hemoglobin	g/dl	Advia 120
Hematocrit	%	Advia 120
Platelets	$\times 10^3/\mu l$	Advia 120
MCV	fl	Advia 120
MCH	pg	Calculated
MCHC	g/dL	Calculated
Differential leukocyte count	$\times 10^3/\mu l$	Advia 120
% reticulocytes	%	Calculated
Absolute reticulocytes	$\times 10^6/\mu l$	Advia 120
Serum chemistry		
Calcium	mg/dl	Cresophthalein complexone
Phosphorus	mg/dl	Phosphomolybdate-UV
Glucose	mg/dl	Hexokinase
BUN	mg/dl	Urease with GLDH
Creatinine	mg/dl	Kinetic alkaline picrate
Total cholesterol	mg/dl	Enzymatic esterase-oxidase
Total protein	g/dl	Biuret
Albumin	g/dl	Dye binding (BCG)
ALP	U/l	Kinetic r-nitrophenyl-phosphate
Globulin	g/dl	Calculated
AST	U/l	Kinetic NADH oxidation
ALT	U/l	Kinetic NADH oxidation
Na^+	mEq/l	Ion-selective electrode
Cl^-	mEq/l	Ion-selective electrode
K^+	mEq/l	Ion-selective electrode

WBC, white blood cell count; RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; BUN, blood urea nitrogen; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GLDH, Glutamate dehydrogenase; BCG, Bromocresol green.

$$Var(y_{ijk}) = Var(animal_k + e_{ijk}) = \sigma_a^2 + \sigma_e^2$$

and the covariance between any 2 observations, in this case on different treatments, on the same animal is

$$Cov(y_{ijk}, y_{ij'k}) = \sigma_a^2$$

Group assessment. The mixed model

$$y_{ijk} = \mu + group_i + day_j + (group * day)_{ij} + animal_k + e_{ijk}$$

was applied to the saline only data, where μ was the grand mean and *groups*, *days*, and *group*days* were the effects of interest. The *animal* identified the experimental unit on which repeated measurements were observed across the different days for the saline treatment, and *e* was the random error associated with an observed blood parameter reading. The variance and covariance structures are as in the above model with the covariance now being between any 2 observations on different days for the same animal.

Solvents versus saline. The mixed model

$$y_{ijk} = \mu + group_i + day_j + (group * day)_{ij} + trt_k + (day * trt)_{jk} + animal_l + e_{ijkl}$$

was applied to all data, where μ was the grand mean and *groups*, *days*, *group*days*, *trts*, and *day*trts* were the effects of interest. The *animal* identified the experimental unit on which repeated measurements were observed across the different days for each treatment, with the baseline reading represented by day 0, and *e* was the random error associated with an observed blood parameter reading. The variance and covariance structures are as in the above model, with the covariance now being between any two observations on different days for the same animal.

Results

Of the 8 dogs given propylene glycol, 2 vomited a small amount of bile-colored fluid shortly after the infusion ended. One of these dogs also had a small amount of loose stool. Both dogs remained bright and alert, with normal body temperatures and heart and respiratory rates, and had no further episodes of vomiting or diarrhea during the study. Both dogs ate normally when offered food later that day. All dogs remained clinically normal after infusion of the remaining 8 vehicles.

One dog (in Group A) developed an ulcerated skin lesion on its chest during its last vehicle infusion, due to self-trauma of a pre-existing superficial mass. The lesion was cleaned with chlorohexidine scrub (Fort Dodge Animal Health, Fort Dodge, IA); a topical antibiotic ointment containing neomycin, bacitracin, and polymyxin B (Vetro-Biotic, Pharmaderm, Melville, NY) was applied; and the chest was bandaged. After the last blood collection from the dog, the mass was removed.

One dog (in Group B) was diagnosed with unilateral otitis externa during its last vehicle administration, and treatment was initiated using an otic solution containing betamethasone, gentamycin, and clotrimizole (Otomax, Schering-Plough Animal Health, Union, NJ). This dog also had a recent history of interdigital cysts, but they were quiescent during this study.

Group mean values for each hematology and clinical chemistry parameter, for all of the vehicles tested at all time points, remained within established normal ranges throughout the study. In addition, individual values for most dogs remained within established normal ranges (Table 2) throughout the study. The only noteworthy exceptions were 3 clinical chemistry values for the dog that developed unilateral otitis externa. Total protein (7.4 to 7.9 g/dl; normal range, 5.1 to 7.1 g/dl) and

Table 2. Hematologic and serum clinical chemistry variables: normal values (range)

Variable	Units	Duncan[a]	MF[b]	In-house laboratory
Hematology				
WBC	$\times 10^3/\mu l$	5.0–14.1	6.0–19.5	4.80–22
RBC	$\times 10^6/\mu l$	4.95–7.87	5.0–8.5	5.88–8.29
Hemoglobin	g/dl	11.9–18.9	11.9–18.9	13.6–19.7
Hematocrit	%	35–57	34–58	39.9–56
Platelets	$\times 10^3/\mu l$	211–621	140–850	178–587
MCV	fl	66–77	60–80	60–73
MCH	pg	21.0–26.2	17.5–28	19.8–25.6
MCHC	g/dl	32.0–36.3	25–38	32.1–35.3
Neutrophils	$\times 10^3/\mu l$	2.9–12.0	1.8–16.6	2.86–17.06
Lymphocytes	$\times 10^3/\mu l$	0.4–2.9	0.7–11.7	1.38–4.49
Monocytes	$\times 10^3/\mu l$	0.1–1.4	0.0–2.7	0.09–1.26
Eosinophils	$\times 10^3/\mu l$	0.0–1.3	0.0–1.9	0.04–0.96
Basophils	$\times 10^3/\mu l$	0.0–0.14	0.0–0.6	0.0–0.17
% reticulocytes	%	0.0–1.0	NA	0.2–2.3
Absolute reticulocytes	$\times 10^6/\mu l$	< 0.080	NA	0.011–0.175
Serum chemistry				
Calcium	mg/dl	9.1–11.7	7.2–12.8	9.0–11.2
Phosphorus	mg/dl	2.9–5.3	3.3–6.0	1.8–4.6
Glucose	mg/dl	76–119	60–120	69–113
BUN	mg/dl	8–28	8–30	9–26
Creatinine	mg/dl	0.5–1.7	0.5–1.3	0.7–1.1
Total cholesterol	mg/dl	135–278	124–335	92–226
Total protein	g/dl	5.4–7.5	5.6–7.1	5.1–6.4
Albumin	g/dl	2.3–3.1	not available	2.4–3.6
ALP	U/l	1–114	12–122	17–152
Globulin	g/dl	2.7–4.4	1.9–3.6	2.2–3.3
AST	U/l	13–15	16–50	16–52
ALT	U/l	10–109	25–106	14–255
Na^+	mEq/l	142–152	142–151	144–153
Cl^-	mEq/l	110–124	107–117	104–115
K^+	mEq/l	2.9–5.3	3.9–5.3	3.8–4.9

WBC, white blood cell count; RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; BUN, blood urea nitrogen; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[a]Normal values from J. R. Duncan, 1994.[3]
[b]Normal reference values from Marshall Farms (for Beagles 12 months of age and older).

globulin levels (4.9 to 5.1 g/dl; normal range, 1.9 to 3.6 g/dl) were elevated at the beginning of the dog's 3rd vehicle infusion (DMA, time point 0) and remained elevated throughout the remainder of the study. This dog also had minimally elevated alkaline phosphatase levels at the beginning of its 1st vehicle infusion (10% DMSO, time point 0) that remained elevated throughout the remainder of the study (118 to 167 U/l; normal range, 1 to 114 U/l).

Baseline analysis of Day 0 saline data indicated no significant differences between Groups A and B. Mean values for 2 serum chemistry parameters and 13 hematology parameters were statistically different ($P < 0.05$) from their comparable saline controls (Table 3).

Discussion

The goal of this study was to determine whether the described vehicles caused any adverse clinical or physiologic effects when administered to dogs. Except for 2 mild cases of vomiting and 1 mild case of loose stool, all dogs remained free of obvious vehicle-induced clinical abnormalities throughout this study. In addition, there were no adverse, acute physiologic effects on hematology and serum chemistry parameters. Although some of the vehicles used in this study have been reported to be highly hemolytic solvents—particularly propylene glycol, DMSO, and NMP[12]—we observed no intravascular hemolysis in any dog during this study.

Although 'statistically significant' differences were found for a few parameters when comparing vehicle data with comparable saline means, there were no biologically relevant differences. Only 1 dog had elevated serum chemistry values that were considered biologically relevant as compared with established normal values. However, the elevated alkaline phosphatase, globulin, and total protein levels in that dog most likely were due to its underlying ear infection and recent history of interdigital cysts rather than to administration of any of the test vehicles. Elevated serum alkaline phosphatase can be caused by a number of conditions, including cholestasis, bone disease, and neoplasia, or by the administration of corticosteroids and other nonsteroidal drugs.[3] However, it is also not uncommon to have unexplained elevations in alkaline phosphatase, as the enzyme has high sensitivity and low specificity and is widely distributed throughout the body.[3] The elevations seen in this dog were relatively minimal. Mild elevations in globulin levels are usually due to acute-phase proteins, indicative of trauma or inflammation, and the dog's history is the most likely cause of the elevations noted in this study.

This study does not answer all the questions surrounding the use of vehicles in dogs. Dogs often remain on DMPK protocols for years and thus potentially are exposed to a large number of organic solvents. The dogs used in this study were naïve and to our knowledge had not received any of these vehicles prior to this experiment. This study was designed to examine the acute

Table 3. Canine hematology and clinical chemistry values statistically different from saline controls

Parameter	Units	Vehicle	Day	Vehicle mean[a]	Saline mean[a]	P[b]
BUN	mg/dl	β-cyclodextrin	1	20.4	15.6	0.0242
Glucose	mg/dl	NMP	7	88.3	92.3	0.0027
Hemoglobin	g/dl	Ethyl alcohol	7	17.9	16.9	0.0448
Hematocrit	%	Ethyl alcohol	7	54.9	48.8	<0.0001
MCH	pg	Glycofurol	1	23.5	23.2	0.0003
MCH	pg	DMSO	1	23.7	23.2	0.004
MCHC	g/dl	Glycofurol	1	34.5	34.2	0.0297
MCHC	g/dl	Glycofurol	7	34.9	34.7	0.0022
MCHC	g/dl	Propylene glycol	1	35.1	34.2	0.0224
MCHC	g/dl	Ethyl alcohol	7	32.6	34.7	<0.0001
MCV	fl	DMSO	1	68.7	67.8	0.0004
MCV	fl	DMA	1	70.1	67.8	<0.0001
MCV	fl	DMA	7	68.2	67.4	0.0311
MCV	fl	Ethyl alcohol	1	68.6	67.8	0.0287
MCV	fl	Ethyl alcohol	7	72.9	67.4	<0.0001

BUN, blood urea nitrogen; NMP, N-methyl-2-pyrrolidone; MCH, mean corpuscular hemoglobin; DMSO, dimethyl sulfoxide; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; DMA, dimethylacetamide.
[a]All means reported are least square means.
[b]Sidak–Holm-adjusted P values.

affects of a single vehicle, given once, intravenously. Additional studies are needed to determine the chronic affects of vehicle administration, both alone and in combination or succession with other vehicles.

We conclude that at the dose, route, and manner described, all of the vehicles tested in this study are clinically safe to use and have no acute effects on hematology or serum chemistry parameters. These results will prove useful in establishing guidelines on vehicles acceptable for use in dogs.

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