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Review Article

Pharmacogenetic and Metabolic Differences Between Dog Breeds: Their Impact on Canine Medicine and the Use of the Dog as a Preclinical Animal Model

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Abstract. There is limited information describing species related pharmacogenetic differences in animals. Despite the lack of genetic information in veterinary medicine, breed specific responses to endogenous and exogenous substances have been reported across many species. This finding underscores the importance of obtaining insight into the genotypic and phenotypic variation present across breeds. This article provides a summary of the literature pertaining to canine breed differences in physiology, drug response, drug pharmacokinetics, and metabolic idiosyncrasies. The existing knowledge of pedigrees and the known phenotypes and genotypes of dogs provides important information for determining mode of inheritance, penetration, and other major characteristics of heritable traits. Understanding these breed differences will improve canine population predictions (for canine drug products) and may be of value when extrapolating toxicology data from dogs to humans.

KEY WORDS: bioavailability; breed-related differences; canine pharmacodynamics; canine pharmacogenetics; canine pharmacokinetics; drug response; population diversity.

INTRODUCTION

There is a limited body of work describing species related pharmacogenetic differences in animals, despite the explosion of pharmacogenetic information in humans (see AAPS Pharmacogenetics/Pharmacogenomics Virtual Journal, http://www.aapsj.org/theme_issues/virtual/index.asp). Published literature confirms that there is little difference between human and veterinary species in regard to the magnitude of inter-subject variability in drug pharmacokinetics and pharmacodynamics. Despite the lack of genetic information in veterinary medicine, breed specific differences in response to endogenous and exogenous substances have been reported across a range of species, including cattle (1), sheep (2), chickens (3), pigs (4), and dogs (as described below).

Whether the goal is to maximize the human prediction potential of data obtained in dogs or to improve our ability to extrapolate limited canine safety and effectiveness data to the entire canine population, it is important to appreciate the genotypic and phenotypic variation that is present across breeds. With this objective in mind, the Animal Pharmaceutics and Technology Focus Group of the American Association of Pharmaceutical Scientists (AAPS) launched a working group to examine the breed-specific idiosyncrasies in physiology and metabolism that could influence both the use of dogs as an animal model for human drug research and the development of pharmaceuticals for use in dogs. This working group provided discussion and articles that were used in the development of a database that focused on phenotypic and metabolic differences observed across breeds of dogs.

This article summarizes the published work on canine breed differences in physiology and pharmacology. It reflects an effort to establish a library of published literature that describes differences in drug response, drug pharmacokinetics, or metabolic idiosyncrasies that can lead to either inconsistencies in the results of studies conducted in dogs (across breeds) or failure to adequately predict breed-specific differences that could lead to safety or effectiveness concerns when products are administered to dogs.

BREED OVERVIEW

A breed is defined as a group of organisms having common ancestors and certain distinguishable characteristics developed by artificial selection and maintained by controlled propagation (5). Across domestic animal species, some of the greatest physical diversity may be found across breeds of dog. For example, canine body weights range from approximately 4 lb for the Chihuahua to over 200 lb for the Great Dane, St. Bernard, and Irish Wolfhound. Recently, a portion of the size difference between breeds of dogs has been traced to a single gene encoding for insulin-like growth factor 1 (IGF-1). A single IGF-1 single-nucleotide polymorphism haplotype was found to be common to all small breeds and practically absent from giant breeds (6).

There are over 400 breeds of dogs recognized worldwide and 156 breeds recognized by the American Kennel Club

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(AKC; http://www.akc.org/reg/dogreg_stats.cfm). The work of Parker *et al.* (7,8) reinforces the position that some canine breeds are more closely related than others. A diagram of the canine family tree is provided in Fig. 1.

Although most modern canine breeds have existed for fewer than 400 years, each has its own distinctive genetic characteristics. Fixation of the phenotypic appearance and the mating of closely related individuals have resulted in breed-specific disease patterns and great variations in life expectancy. This size differences between breeds can also be associated with deleterious physical consequences. In a study of over 52,000 dogs, the likelihood of reaching 10 years of age was greater than 85% for Poodles, 75% for mongrels and Beagles, and only 30% for Bernese Mountain Dogs (9). Of course, size is only one of many factors associated with the average life span of dogs. Unrelated to their size, the shortened average lifespan of Cavalier King Charles Spaniels, Irish Wolfhounds, and Great Danes, for instance is due to a high risk of cardiomyopathies (10). Indeed, many breeds display an excess of particular disease as has been cataloged by Sargan and colleagues (11).

With this great magnitude of genetic diversity, it is not surprising that there are both metabolic and physiologic idiosyncrasies that can influence not only the propensity for certain disease conditions, but also drug pharmacokinetics and the characteristics of responses to xenobiotics.

GENETICS AND BREED

Despite the advent of modern genetic methods, pedigree analysis remains an essential, and in some cases the only technique available to analyze the inheritance of genetic defects or desirable traits. Coupling this existing knowledge of pedigrees with known phenotypes and genotypes of the dog provides important information for determining mode of inheritance, penetration, and other major characteristics of a heritable trait. However, for dogs that do not have their pedigree information available, Parker *et al.* (7,8) revealed that one can identify the diversity of breeds, or lack thereof, in an individual dog through DNA analysis.

The genetic sequence of the dog, from one female Boxer, became publicly available in July, 2004 (12). The draft sequence can be accessed online (13–15). The University of California, Santa Cruz published more information on the search capabilities of the sequence and annotation data in the genome databases (16). Just as important is the 1.5× sequence of the standard poodle, which became available in 2003 and provided an additional set of chromosomes for evaluation (17).

Based upon work conducted, at the Fred Hutchinson Cancer Research Center in Seattle WA, dogs can be correctly assigned to their breed of origin 99% of the time through the use of DNA analysis (18). This breed identification is based on the differences in DNA markers between breeds and microsatellite loci (8). This information can be used in turn to determine if a dog may be predisposed to certain genetically based diseases. A genetic test is commercially available through Mars Veterinary (19). Knowledge of breed predisposition can be especially important if a molecular test is not available for the trait of interest.

Other uses for DNA markers may include the identification of genetic diseases or metabolic differences that can be localized within related groups of breeds. Alternatively, genotyping could identify individuals with specific genetic mutations that can affect therapeutic decisions. Searchable databases of canine markers and chromosome maps are available on the Canine Genetics Research Projects page at

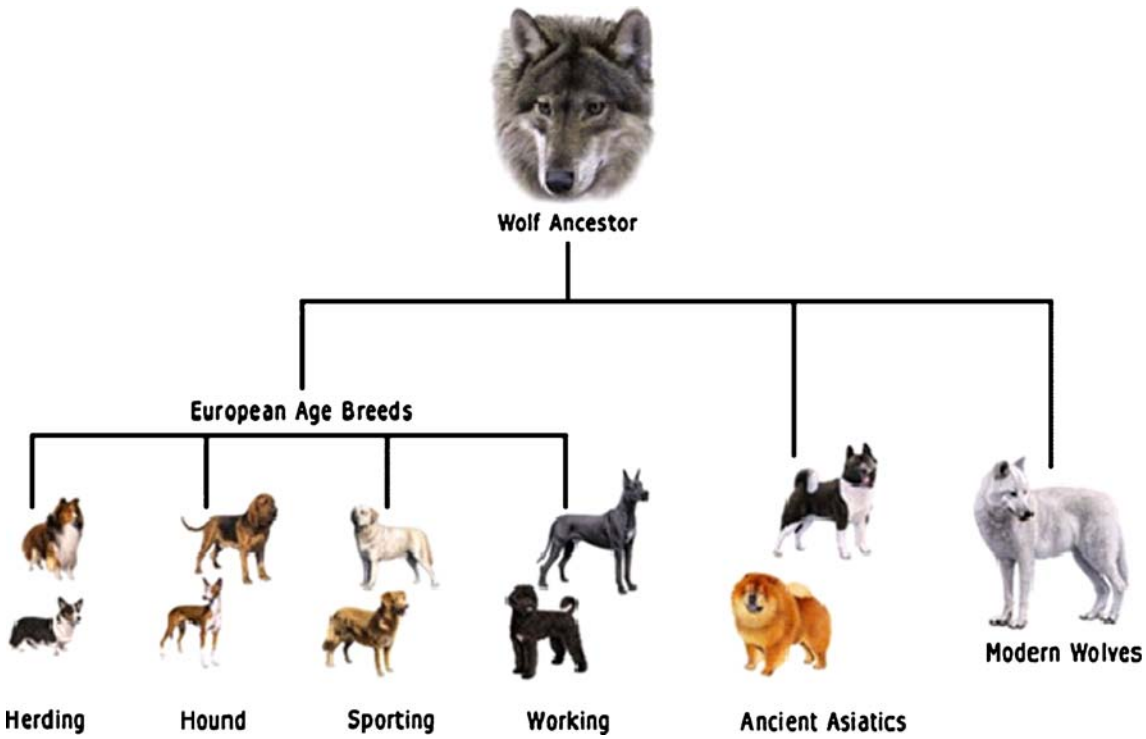

Fig. 1. Dog History Tree. [Reproduced with permission from Mars Veterinary]

the Universite Rennes (20). In addition, a canine SNP database by chromosome and breed is available on-line (21). An updated database is available at the National Human Genome Research Institute (NHGRI) and at the Broad Institute (21,22). The ultimate goal is to go beyond markers and have a complete genome map that permits the identification of traits directly from the DNA code of an individual animal.

SIMILARITIES BETWEEN HUMAN AND CANINE GENOME

Interest in the canine genome project is sparked by the insight it provides into the relationship between human genetics and disease. In their 2005 review of the canine genome, Ostrander and Wayne describe how understanding genetic diseases in dogs greatly impacts the understanding of important diseases in both dogs and humans (23). Among the many disease conditions being investigated, cancer, deafness, heart disease, blindness, and epilepsy are examples of conditions well suited for genetic mapping in dogs (24).

Comparisons between the domesticated dog and human genomes reveal distinct differences as well as some important similarities (Tables I and II).

While the information presented in Tables I and II is based upon an analysis of the complete genome of a single dog (Boxer), a partial genome analysis of a male Standard Poodle revealed similar findings in the comparison of the human and dog genomes (17). Finally, when examining the base pairs comprising a representative sample of canine genes, compositional correlations between the species are also identified (25). Therefore, the differences between the human and canine genomes exist primarily at a superficial level, and a detailed analysis of the genomes reveals that the two genomes have many significant similarities.

The similarities of the genomes are also reflected in the identification of genetic defects associated with disease. Over 360 genetic disorders observed in humans have also been identified in dogs. Almost all of these defects were first identified in humans and involve a mutation of the same or an analogous gene in the dog (26).

An understanding of the canine genome, together with our knowledge of disease incidence within a breed, facilitates an understanding of the genetic basis of disease within the human population. Points to consider are:

1. Does disease incidence differ by breed?
2. Do we see a breed-related difference in the frequency of a specific mutation of a gene(s) potentially related to the disease?
3. Can we establish this defect(s) as the cause of the disease in dogs?
4. Is there a correlation between the disease as expressed in humans and dogs?
5. Is there an analogous gene in humans?

Table I. Differences in Human and Canine Genome (12)

	Differences	
	Canine	Human
Diploid number	78	46
Number of base pairs (Gb)	2.4	2.9
Predicted number of genes	19,300	22,000

Gene duplications in humans lineage occurs approximately twice as frequently as that observed in the canine lineage

Table II. Similarities in Human and Canine Genome (12)

Similarities
Ninety-four percent of the dog genome is in the same order within individual chromosomes (synteny) as seen in the human, mouse, and rat
The majority of the predicted genes in the canine genome are homologues of known human genes
The disparity between the numbers of genes in humans and dogs may become smaller as the techniques for identifying functional genes from the predicted genes improve
Although the number and frequency of gene duplications specific to each lineage differ, the duplications occur in similar gene clusters, including genes involved in adaptive immunity, innate immunity, chemosensation, and reproduction

In addition to the genetic similarities identified between humans and dogs, the modern dog offers key advantages over other animal systems because of constraints placed on its diversity. In addition, catastrophic events such as the two world wars and the American depression have reduced the effective breeding stock. Therefore, the purebred dogs of today represent a limited genetic pool, with disease predispositions that derive from one or a small number of recent genetic founders (24).

From a population perspective, the mating of closely related individuals to propagate selected characteristics has resulted in a greater degree of genetic diversity between dog breeds as compared to that observed between human ethnic groups. For example, the tendency towards specific metabolic diseases appears to follow breed lines. However, unlike humans where inborn errors of metabolism are generally attributable to many different mutations in a particular gene, in dogs (and cats), the same mutation is generally responsible for the specific disease within a particular breed (27). While only 5% to 10% of human genetic variation has been shown to be associated with populations or races, 27% of genetic variation in dogs is associated with differences in breed (7). Since at least 50% of the defined hereditary diseases in dogs have significant breed-specific aggregations (26), there is a greater likelihood of identifying genetic links in humans by utilizing known breed differences in dogs.

GENETICALLY RELATED DISEASES IN THE DOG

Breed differences in the incidence of specific diseases are well recognized in veterinary medicine (28).

There are over 370 inherited disorders described in the purebred dog population. Of the identified disorders in which the mode of inheritance is known with reasonable certainty, more than half are identified as autosomal recessive (26). In contrast, some important heritable defects, such as hip dysplasia and osteoarthritis in dogs, involve multiple genes and environmental influences (29). The predominance of diseases associated with autosomal recessive traits can be

attributed to the breeding and selection patterns used by breeders to propagate specific traits.

Several databases on heritable diseases and traits in dogs and other species are available on the internet:

- Online Mendelian Inheritance in Animals (OMIA) is a database of genes, inherited disorders and traits in more than 135 animal species (other than human and mouse) (30). The database contains textual information and references, as well as links to relevant PubMed and gene records at the National Center for Biotechnology Information (NCBI). The NCBI also has a dedicated webpage to dog genome resources (31).
- The Inherited Diseases in Dogs (IDID) web site contains a database of diseases/conditions of pure bred dogs which are likely to be transmitted completely or partially through a genetic mechanism (11,32). The site also lists the testing agencies (UK and USA) where DNA testing is available for a given disease.
- The Canine Inherited Disorders Database (CIDD) was developed to reduce the incidence of inherited disorders in dogs by providing information to owners and breeders, and to facilitate the best management possible of these conditions by providing current information to veterinarians (33). The database is searchable by breed and organ systems.
- The Listing of Inherited Disorders in Animals (LIDA) is designed to gather, collate and disseminate data on the prevalence of inherited disorders among Australian dogs (34). The database is searchable by breed group, breed, and organ systems.
- The NCBI website offers numerous databases including Online Mendelian Inheritance in Man (OMIM), a database of human genes and genetic disorders (12).

The identification of specific gene mutations and gene markers provides information on, and the ability to test for, multiple genetic diseases. Therefore, molecular genetic testing is a rapidly evolving science within veterinary medicine (35). Bannasch and Hughes (36) compiled a list of the available commercial tests and the laboratory contact information. Currently, there are over 100 DNA-based tests for inherited diseases and traits in dogs. For example, a test is available to determine the presence of MDR-1 mutations in Collies; this mutation results in increased toxicity when these dogs are administered certain *p*-glycoprotein substrates such as ivermectin (37,38).

In the future, validated microarray technology will be available to screen dogs for polymorphisms in metabolic enzymes in a single test. This technology already exists for testing certain CYP polymorphisms in humans. For example, the FDA approved AmpliChip CYP450 Test which identifies a human patient's CYP2D6 and CYP2C19 genotype from genomic DNA extracted from a whole blood sample (39).

BREED-RELATED DIFFERENCES IN PHARMACOKINETICS AND PHARMACODYNAMICS

The genetic variation that exists between breeds of dogs results in a risk of breed-related differences in the effectiveness and toxicological responses to drugs. This potential source of population variability needs to be considered in the design and interpretation of studies of the canine physiology and drug pharmacology.

A list of identified breed-specific idiosyncrasies that can influence drug absorption and metabolism are provided in Tables III and IV (40–54). Table III includes recognized metabolic idiosyncrasies and Table IV includes physiologic idiosyncrasies. Abbreviations are provided at the end of each table.

Breed-related differences can influence drug pharmacokinetics and pharmacodynamics. Therefore, there can be discordance in the dose-response relationship across breeds. For example, differences, such as the lower percent body fat seen in the Greyhound, can lead to a lower than expected volume of distribution for lipophilic compounds (43). This lower percent body fat can result in higher serum drug concentrations as compared to that seen in breeds with a higher percent body fat. Accordingly, Greyhounds are associated with a greater risk of toxicity for lipophilic compounds.

Large breed dogs also tend to be predisposed to sulfonamide polyarthropathy. Reports of sulfonamide arthropathy have been reported in Labrador retrievers, Golden retrievers, Great Danes, Dalmations, Giant Schnauzers, Briards, Weimaraners, Irish setters, Flat coated retrievers, Gordon setters, Springer spaniels, German short haired pointers, and Airedales, with only two cases reported in smaller dogs (one Cocker spaniel and one Pekingese) (55). This tendency tends to be particular prevalent among Doberman Pinschers, where protein-losing nephropathy, leukopenia, and modest thrombocytopenia have also been observed (55). The basis for the Doberman's predisposition to sulfonamide toxicity may reflect the limited capacity of this breed to detoxify the hydroxylamine metabolites of the sulfonamides (56).

In another example, the size of a dog appears to influence growth rate, vitamin D3 metabolism and circulating levels of IGF-1 (which indirectly reflects the levels of growth hormone) (57). Great Danes (GD) have a statistically significantly greater rate of growth as compared to Miniature Poodles, (MP) significantly greater plasma concentrations of growth hormone (approximately 20 *versus* 7 μg/l for the GD and MP, respectively at 7 weeks of age) and IGF-1 (approximately 400 *versus* 150 μg/l for the GD *versus* MP, respectively at 16 weeks of age), and a significantly lower clearance of vitamin D3 (approximately 0.625 l/kg per day *versus* 0.3 l/kg per day for the GD and MP, respectively at 19 weeks of age). GD's also have a significantly higher rate of renal reabsorption of inorganic phosphate as compared to the MP and a significantly greater rate of bone turnover and resorption. Furthermore, the GD has a significantly higher frequency of growth plate irregularities, which may be due to higher levels of calcitonin and/or lower concentrations of vitamin D3 metabolites (relative to the MP). It is also noteworthy that in adult dogs, plasma IGF-1 concentrations are reported to be related to body size (58,59) but that intestinal calcium absorption does not appear to be correlated with body size (although it does appear to be correlated with dog age) (60).

Considering the potential impact of breed on drug pharmacokinetics and pharmacodynamics, studies employing similar protocols but different breeds can lead to inconsistent

Table III. Examples of Breed Related Metabolic Differences

Breed	Enzyme or Gene Linked to Finding	Genetic Finding/Metabolic Effects
Beagle	CYP1A	Polymorphism/genotypic variation in CYP1A2 activity (due to differences in enzyme expression). Poor metabolizers and extensive metabolizers result in significant polymorphic hydroxylation of a novel benzodiazepine (40,41)
Greyhound	CYP2B11	Deficient in CYP2B11. Canine CYP2B11 is responsible for propofol hydroxylation in dogs (42,43). CYP2B11 may be orthologue to human CYP2B6.
Mixed Breeds	CYP2B11	14-fold variance in CYP2B11 activity in mixed breed dogs. Mixed breed dogs tended to span range of Vmax seen in Beagles (high) and Greyhounds (low) (42,43)
All breeds	CYP2C	CYP2C is polymorphic in dogs. Two isoforms identified—CYP2C21 in all evaluated dogs (25/25). CYP2C41 only found in 14–16% of tested dogs. CYP2C41 appears neither breed or gender specific (43)
Beagle	CYP2D15	Significant pharmacogenetic variation in gene encoding CYP2D15 (counterpart to human CYP2D6). Other undefined CYPs may also be involved. Significant pharmacogenetic variation in celecoxib metabolism in purebred Beagles. Celecoxib is primarily a CYP2D15 substrate in dogs. Extensive metabolizers (about 50% of those tested) have an elimination half-life of 1.5–2 h. Poor metabolizers have an elimination half-life of approximately 5 h. No gender differences observed (45)
All breeds	*N*-Acetyltransferase	Absent in all dogs, due to loss of both *N*-acetyltransferase genes in canids. *N*-acetylation detoxifie sulfonamides, procainamide, dapsone, isoniazid, and hydralazine. This absence may predispose dogs to sulfonamide hypersensitivity and adverse effects from hydralazine and dapsone (46)
Labrador Retriever	TPMT	Statistically significantly higher RBC thiopurine Retriever *S*-methyltransferase activity than other breeds. TPMT detoxifies azathioprine metabolites. Low TPMT activity puts animals (or human) at risk for toxicity when using thiopurines. RBC TPMT activity not correlated with age or gender. Nine unequivocal haplotypes identified in dogs (47)
Cocker Spaniel	TPMT	Tended to have lower (but not significant) TPMT activity as compared to other breeds. More testing is needed (47)
Various breeds	TPMT	Survey of TPMT activity in 177 dogs found >ninefold range in TPMT activity across dogs of different breeds (47)
Bedlington Terrier	Murr-1	Copper storage hepatopathy due to defects in the Terrier Murr-1 gene. Disease is autosomal recessive. Affected dogs have chronic hepatitis resulting from a primary defect in copper excretion and abnormal copper retention in the hepatocytes. May effect drugs metabolized by the liver (48–50)
Boxer		Sensitive to acepromazine (42)

Abbreviations:
CYP: Cytochrome P450 oxidative enzymes. For example, the nomenclature, CYP2D6 refers to the enzyme (CYP), gene family (e.g., 2) subfamily (D), and individual gene (6)
Vmax: Maximum velocity of an enzymatic reaction
RBC: Red blood cell
TPMT: Thiopurine methyltransferase

results. This can have important consequences in studies where new medications are being evaluated for use in dogs, when determining appropriate medications or dosages in veterinary practice, or when using the dog as a preclinical species for human drug development. For example:

1. Platelet aggregation response to arachidonic acid (*in vitro* assay) was highly dependent upon breed, with some breeds having only a small proportion of individuals exhibiting an irreversible response (e.g., Greyhounds and Beagles) while other breeds (Scottish Terrier) were associated with the majority of individuals having an irreversible response (61). Therefore, breed differences in platelet aggregation responses to certain drugs may be an important consideration when using the dog as a model for platelet-related cardiovascular disorders.
2. The Welsh Corgi is known to have an autosomal recessive severe combined immunodeficiency. Male Weimaraners are predisposed to a neutrophil function defect with a primary or secondary reduction in circulating IgG (62). Affected animals of both of these breeds

Table IV. Examples of Breed Related Physiologic Differences

Breed	Organ System	Genetic Finding
Greyhound	Cardiovascular	Higher resting blood pressure and higher packed red cell volume than other breeds (48,51)
Japanese Akita	Hematologic	RBCs contain more potassium than other breeds (48)
Beagle (laboratory)	Hematologic	Primary idiopathic hyperlipemia (familial) (48)
Beagle (laboratory)	Hematologic	Factor VII deficiency; autosomal dominant trait, heterozygotes are asymptomatic (48)
Cairn Terrier	Hematologic	Lower LDL and higher HDL than larger breeds. Total cholesterol and triglycerides did not vary across breeds, but HDL and LDL levels were breed-dependent (52)
Labrador Retriever	Hematologic	Higher LDL and lower HDL than smaller breeds (52)
Beagle (laboratory)	Immune	Severe combined immunodeficiency, X-linked trait (48)
Beagle	Immune	Lymphocytic thyroiditis, causing hypothyroidism
Japanese Akita	CNS	Narcolepsy, cataplexy, and epilepsy (48) inheritance suspected
Greyhound	Endocrine	Lower free T4 and total T4 than other breeds (48)
Basenji	Renal	Faconi syndrome; poor tubular resorption of glucose, amino acids, and phosphate. Most affected dogs present at 1–5 years of age with PU/PD (48)
Sighthounds	Musculoskeletal	Lower Vdss of lipophilic compounds (e.g., propofol) Likely reflects the lower % body fat compared to other breeds (42)
Large *vs* Small Breeds	Gastrointestinal	Body size and breed related differences in small breeds pyloric sieving, GI transit time, fecal quality, and "leakiness" of the tight junctions (see discussion later in this review)
	Renal	GFR, expressed per kg body weight, tends to be inversely correlated to body size (53). Accordingly, plasma creatinine values tend to be higher in large (>30 kg) as compared to small (>10 kg) breed dogs (54)

Abbreviations:
LDL: Low density lipoprotein
HDL: High density lipoprotein
CNS: Central nervous system
PU: Polyuria
PD: Polydipsia
GI: Gastrointestinal
GFR: Glomerular filtration rate

are predisposed to recurrent infections and would likely reveal reduced effectiveness of antimicrobial drugs, especially drugs that work within white blood cells.

3. The risk of ibuprofen-related GI ulceration is low for Labrador Retrievers but is very high for German Shepherds (63).
4. Several surveys have been conducted to ascertain the occurrence of spontaneous tumors in various canine breeds (64–66). In addition, several sources provide an overview of relative risk of developing malignancies as a function of breed (21,67,68). Despite the absence of detailed epidemiological data, there is a wealth of circumstantial evidence supporting a relationship between breed and the relative risk of certain cancers.
   (a) The Boxer has a significantly greater risk of developing malignant melanoma as compared to other breeds. In contrast, the Chihuahua rarely presents with malignant melanoma (64). Melanoma is most common in older dogs with dark pigmented skin and accounts for between 5% and 7% of all canine skin tumors (69).
   (b) In terms of the risk of developing mammary cancers, it is sevenfold higher in intact as compared to neutered females, and the pure-bred mammary cancer rate is higher in each age group as compared to cross-bred females ($p<0.025$) (65).
   (c) Osteosarcoma is the most common type of primary bone cancer accounting for up to 85% of tumors that originate in the skeletal system. Of total canine malignancies, osteosarcoma accounts for about 5%. The giant breeds (for example, Great Danes, Mastiffs, Bernese Mountain Dogs, and Irish Wolfhounds) are particularly susceptible. Large breeds such as Rottweilers, Labradors, Golden Retrievers, Shepherds, Dobermans, Weimaraners, Greyhounds and Boxers are also at an increased risk (70,71).
5. The use of dogs in studies to assess the human carcinogenicity potential for contraceptive steroids and other compounds may be problematic because certain breeds tend to naturally be more prone to malignant neoplasia (72). Furthermore, while Beagles are concluded to be an adequate model for testing drug carcinogenicity, particularly for contraceptive steroids, certain strains of Beagle may present a higher risk than others (72,73).

Differences across breeds also exist in the dose-response relationship to anticholinergic and prokinetic compounds. Two anticholinergic drugs (atropine and glycopyrrolate) and

two prokinetic drugs (metoclopramide and cisapride) were evaluated in four Beagles and four Labrador Retrievers (74). In Beagles, low doses of atropine [0.02 mg/kg via intramuscular (IM) injection] and glycopyrrolate (0.005 mg/kg by IM injection) completely inhibited gastric motility for at least 30 min, while higher doses (0.04 and 0.01 mg/kg for atropine and glycopyrrolate, respectively) resulted in a cessation of activity for more than 3 h. In Labradors, the effects of glycopyrrolate lasted at least 6-h, regardless of dose, and the effects of atropine lasted for approximately 3-h, regardless of dose. With respect to the prokinetic agents, the increase in amplitude of gastric contractions of Beagles receiving a low dose of metoclopramide (0.3 mg/kg IM) or cisapride (0.2 mg/kg IM) was significant, but higher doses paradoxically caused a lesser increase in the amplitude of gastric contractions. In Labradors, both medications, mainly at higher doses, resulted in an increase in the amplitude of intestinal contraction. However, the low dose of cisapride had no effect, and a low dose of metoclopramide exerted only a transient effect. Cisapride did not affect the frequency of antral contractions in either Beagles or Labradors. In Beagles, metoclopramide resulted in a dose-related increase in the frequency of contraction. Metoclopramide did not increase the frequency of contraction in Labradors.

BREED DIFFERENCES IN GI PHYSIOLOGY

The GI tract of large breed dogs (e.g. 60 kg) comprises 2.8% of their total body weight. In contrast, it comprises 7% of the total body weight of small breed dogs (e.g. 5 kg) (75). There are also marked differences in fecal moisture content where giant breeds tend to have greater moisture content and a higher frequency of loose stools as compared to those of small breed dogs (76). Such breed-related differences could reflect dissimilarities in GI transit time, intestinal fermentation, diet, metabolism or drug absorption. For example, fecal quality is softer in dogs fed canned diets as compared to those fed dry diets, and this food-related influence on fecal consistency is more pronounced in German Shorthair Pointers and German Shepherds as compared to Beagles, suggesting a breed-related difference in the way that diets are handled (77).

The relationship between breed and fecal quality could also reflect a relationship between breed and GI transit time. Using radiopaque markers (1.5 mm diameter administered in food), 12 week old large breed puppies (e.g. Great Danes) exhibited a significantly longer oro-cecal transit time (OCTT; 3.4 h) as compared to small breed puppies (e.g. Miniature Poodle, 2.5 h). The longer transit time appeared to reflect both a longer gastric emptying time and a longer small intestinal transit time between breeds (78). However, by 60 weeks of age, these breed-related differences in OCTT are significantly reduced (e.g. Great Dane=2.7 h; Miniature Poodle=2.2 h) (79). Although studies involving radiopaque markers do not demonstrate breed-related differences in gastric transit time or small intestinal transit time of adult dogs, investigations involving the ^{12}C-octanoic acid breath test (OABT) do demonstrate a correlation between body size and gastric retention time (80). The inconsistency in study results may reflect the methodologies used for estimating gastric transit time. Estimates of emptying time are highly correlated with particle size (81), and studies involving such particles tend to be associated with greater lag times (gastric retention) (82).

Mean total gastrointestinal transit time (MTT), reflecting time from intake to excretion, was found to be significantly correlated with body size in a different study where transit time was tracked though plastic beads (2 mm diameter) contained in food. In the latter study, MTT increased from 22 h for the Miniature Poodle to 59 h for a Giant Schnauzer (83).

There also appears to be differences in intestinal permeability between dog breeds. The ratio of lactulose (L) to rhamnose (R) reflects the relative absorption occurring across the intestinal tight junction (transcellular transport) *versus* the intestinal surface area (paracellular absorption, which occurs across the cell membrane of the enterocyte). It was noted that marked differences in these ratios occur across breeds, with the L/R ratio being substantially greater in the Greyhound as compared to the Golden Retriever (84).

Breed-related differences exist in the ability for large-sized particles to pass through the pylorus. For example, a tetrahedron-shaped device (2 cm per arm), administered in a capsule after an 18 hour fast, was retained in the 10 kg Beagle for 24 h. However the device was rapidly emptied in the 35 kg American Foxhounds (85). Therefore, the minimum restrictive size for a device retained by the stomach depended on the size of the breed. This observation may be particularly important when considering the development of gastro-retentive devices.

WITHIN-BREED POLYMORPHISM

Breed alone may not provide sufficient information regarding genetic idiosyncrasies. For example, within strains of purebred Beagles, there are subpopulations of fast and slow metabolizers of celecoxib, a finding attributed to genetic polymorphism associated largely with CYP2D15 and CYP3A12 (86). To date, nine canine CYP isoenzymes have been identified through cloning and sequencing. Five of these isoenzymes have been reported as genetically polymorphic (87–90). These include CYP1A2, CYP2C41, CYP2D15, CYP2E1 and CYP3A12. These polymorphisms can lead to differences in drug exposure and could result in strain-related differences in drug responses (the differences in responses may affect the safety and/or effectiveness of the drug). Therefore, breed alone may not provide all the information needed regarding relative factors and genetic traits that may impact a study.

Congenital abnormalities can be linked to a variety of factors including the age of parents at the time of conception, poor nutrition, infections (viral, bacterial, or parasitic), exposure to chemical or other environmental factors, and genetic defects (91). Among the abnormalities of concern, various forms of renal insufficiency have been linked to genetic defects. Renal insufficiencies will impact the pharmacokinetic and toxicological assessment of drugs. Thus, even the use of a single breed does not provide an assurance of pharmacokinetic and pharmacodynamic homogeneity between the study subjects.

Within breeds, there can be genetic subgroups that are associated with distinct metabolic idiosyncrasies. For example, the Poodle can be subdivided into the Standard Poodle (~25 kg),

Miniature Poodle (~15 kg) and Toy Poodle (~5 kg). Across these subgroups, there was a distinct size-related difference in the circulating levels of the potent stimulator of cell replication and DNA and RNA synthesis, IGF-1 (59). These differences in IGF-1 levels and its release in response to IV administration of clonidine (10 μg/kg) were significantly correlated with body weight. None of these three subgroups exhibited a deficiency in growth hormone (GH). This observation led to the suggestion that the apparent GH resistance is related to differences in hepatic endocrine activity.

CONCLUSIONS

Genetic and phenotypic differences across breeds can influence the effect of a dose on product safety and effectiveness. Understanding these breed differences will improve canine population predictions (for canine drug products) and may be of value when extrapolating toxicology data from dogs to humans.. It will also serve to greatly assist our interpretation of data from studies that use dogs as a model for human therapeutics and disease. However, the amount of information on the pharmacokinetic and pharmacodynamic differences that can occur across breeds remains very limited.

The Animal Pharmaceutics and Technology Focus Group of the AAPS hope to continue adding to this body of data to provide a valuable resource for individuals interested in exploring breed differences in dogs and how these differences can influence drug pharmacokinetics and pharmacodynamics. The focus group welcomes additional information that can contribute to this evolving database. To provide additional information or comments please contact Dr. Marilyn Martinez at marilyn.martinez@fda.hhs.gov.

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