Animal Farm: Considerations in Animal Gastrointestinal Physiology and Relevance to Drug Delivery in Humans

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ABSTRACT: "All animals are equal, but some are more equal than others" was the illustrious quote derived from British writer George Orwell's famed work, Animal Farm. Extending beyond the remit of political allegory, however, this statement would appear to hold true for the selection of appropriate animal models to simulate human physiology in preclinical studies. There remain definite gaps in our current knowledge with respect to animal physiology, notably those of intra- and inter-species differences in gastrointestinal (GI) function, which may affect oral drug delivery and absorption. Factors such as cost and availability have often influenced the choice of animal species without clear justification for their similarity to humans, and lack of standardization in techniques employed in past studies using various animals may also have contributed to the generation of contradictory results. As it stands, attempts to identify a single animal species as appropriately representative of human physiology and which may able to adequately simulate human *in vivo* conditions are limited. In this review, we have compiled and critically reviewed data from numerous studies of GI anatomy and physiology of various animal species commonly used in drug delivery modeling, commenting on the appropriateness of these animals for *in vivo* comparison and extrapolation to humans. J Pharm Sci 104:2747–2776, 2015
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INTRODUCTION

The renowned British novelist George Orwell notoriously parodied human behavior and the unfolding events of the Russian Revolution in his 1945 work, "Animal Farm," culminating with the illustrious statement: "All animals are equal, but some are more equal than others." In reality, the relevance of this quote extends to the scientific community, with an ever-prevalent use of animal species to model human responses in preclinical studies. Historically, these uses have ranged from cosmetic product testing to predicting physiological changes under antigravitational conditions and applications in evaluation of *in vivo* drug effects and safety.

Indeed, in terms of the latter, elucidating the mechanisms of drug absorption following oral administration has remained a long-standing goal across a multitude of scientific disciplines, namely, for the purposes of predicting human safety as well as pharmacokinetics and pharmacodynamics for potential drug candidates. *In vitro* studies remain largely inadequate, however, in attempts to simulate the complexities of human gastrointestinal (GI) physiology, which has led to heavy reliance on animal species as intermediary models for evaluation of compounds through *in vitro–in vivo* correlations. These correlations have allowed in more recent years for the assessment of specific physiological and anatomical parameters influencing drug absorption from the gut, toxicological assessment of xenobiotics and vaccines, and perhaps most crucially, enabling dosage estimates to be made when extrapolating data to humans.

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Those animals more commonly used in clinical studies include small rodents such as the rat, mouse, guinea pig, and rabbit, and larger mammals such as the dog, monkey, and pig. Their use is largely down to matters of cost as much as demonstrated similarities with humans for some parameters such as intestinal absorption;[1] rodents being a more popular choice in biomedical research (Fig. 1). However, the use of animals as a "middleman" from which to derive physiological information is hindered by numerous shortcomings owing to intra- and inter-species variability, thus limiting attempts to accurately mimic conditions along the entirety of the GI tract in humans. A systematic comparison of species' differences in GI physiology is also lacking. Indeed, though all mammals have evolved from a common ancestor and thus demonstrate basic phylogenetic similarities, variations in hereditary and environmental adaptations have rendered dissimilarities among them.[2] Consequently, this interspecies diversity in GI physiology has produced marked differences in drug absorption and bioavailability, and as such, the choice of a suitable animal model as well as use of an appropriate experimental design remains crucial to the drug development process. For example, we have previously demonstrated the shortcomings of mice and rats for modeling delivery of pH-responsive ileocolonic formulations owing to their lacking similarity with GI conditions in humans.[3] Likewise, it is not known whether a single animal model provides the best representation of human *in vivo* conditions, or if modeling could be made better by the use of a multitude of different animals intended to reflect different physiological functions. There are many gaps in our present knowledge that support this viewpoint, more so than proven inadequacies for different animal models, and the byzantine interconnections of both animal and human anatomy, physiology, and ecology are yet to be fully understood. Equally, multiple studies measuring the

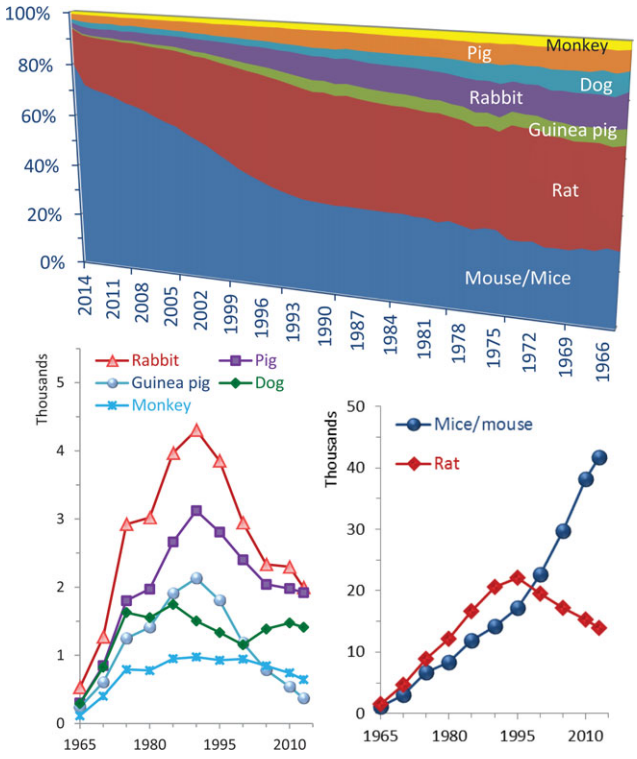

Figure 1. Number of publications in thousands (line plots, bottom) and percentage distribution (area plot, top) indexed in PubMed since 1965 employing use of various animal species in research. Search performed in PubMed using keywords (mice/mouse, rat, monkey, dog, guinea pig, pig, and rabbit), and filters: "title/abstract" and "other animals" under species.

same parameters have produced conflicting results, which suggests that this is either a consequence of the methodology and technique(s) used; the variability between and within animal species; or both. Indeed, we already know that there is much variability in humans alone,[4] and that the same is true for animals, albeit with a far less extensive and detailed understanding of these differences.

In the context of drug delivery, previous reviews have highlighted the implications for animal modeling and the aforementioned anatomical and physiological intra- and inter-species differences that exist.[1,2,5–9] Indeed, as with humans, inter- and intra-variability between animal species is an important consideration for modeling in as much as the demographics of the subject(s) such as age, race, and disease state. Though formulation and compound property influences on drug bioavailability are relatively easy to estimate, the effects of intra- and inter-individual GI variability between species are much more difficult to predict. Figure 2, for instance, provides an interesting visualization of the breadth of differences that exist between animal species and humans in terms of oral drug bioavailability, with data compiled by Musther et al.[10] from 184 different compounds (acidic, basic, neutral, and zwitterionic) from studies published between 1969 and 2012. The figure shows a collated representation of four animal species (mouse, rat, dog, and non-human primate) in addition to humans, and the plots of linear regression separated according to individual species. As can be seen with the dog, rat, and mouse, the correlation with human oral drug bioavailability is very weak (R^2 = 0.384, 0.307, and 0.253, respectively)—only the non-human primate appears to demonstrate a strongly correlating relationship with humans (R^2 = 0.683). Furthermore, interesting trends were observed after classifying the compounds into groups (neutral, acidic,

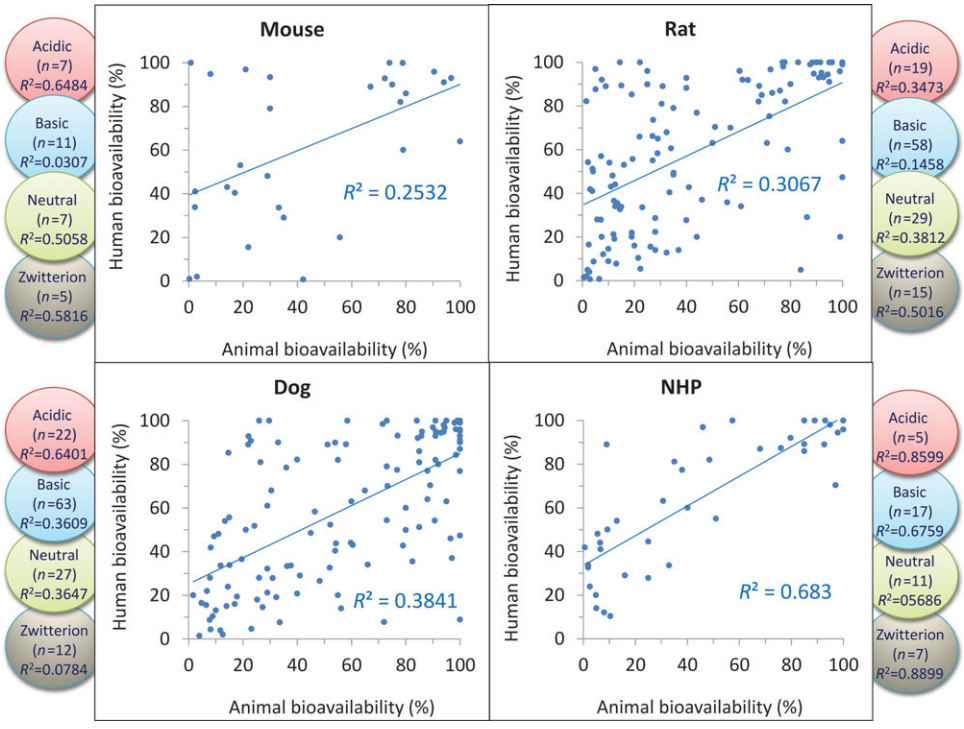

Figure 2. Bioavailability of various drugs in human versus mouse, rat, dog, and non-human primate (NHP). Cumulative trends for all drugs are shown in graphs, whereas bubbles show correlations on classifying drugs in acidic, basic, neutral, or zwitterionic groups. Figure drawn using data from Musther et al.[10]

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