

Translating dosages from animal models to human clinical trials—revisiting body surface area scaling

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ABSTRACT Body surface area (BSA) scaling has been used for prescribing individualized dosages of various drugs and has also been recommended by the U.S. Food and Drug Administration as one method for using data from animal model species to establish safe starting dosages for first-in-human clinical trials. Although BSA conversion equations have been used in certain clinical applications for decades, recent recommendations to use BSA to derive interspecies equivalents for therapeutic dosages of drug and natural products are inappropriate. A thorough review of the literature reveals that BSA conversions are based on antiquated science and have little justification in current translational medicine compared to more advanced allometric and physiologically based pharmacokinetic modeling. Misunderstood and misinterpreted use of BSA conversions may have disastrous consequences, including underdosing leading to abandonment of potentially efficacious investigational drugs, and unexpected deadly adverse events. We aim to demonstrate that recent recommendations for BSA are not appropriate for animal-to-human dosage conversions and use pharmacokinetic data from resveratrol studies to demonstrate how confusion between the “human equivalent dose” and “pharmacologically active dose” can lead to inappropriate dose recommendations. To optimize drug development, future recommendations for interspecies scaling must be scientifically justified using physiologic, pharmacokinetic, and toxicology data rather than simple BSA conversion.—Blanchard, O. L., Smoliga, J. M. Translating dosages from animal models to human clinical trials—revisiting body surface area scaling. *FASEB J.* 29, 1629–1634 (2015). www.fasebj.org

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ONE OF THE GREATEST CHALLENGES in translational medicine is safely and accurately determining the therapeutic human dosage of investigational therapeutics that have been efficacious in laboratory models (1). Despite a wealth of research on pharmacokinetic prediction, suboptimal dosage

and unanticipated adverse events can occur when exploring investigational therapeutics in humans (1, 2). In recent years, the 2008 paper by Reagan-Shaw *et al.* (3) that advocates for body surface area (BSA) scaling between species has emerged as a reference for developing human equivalent dosages from laboratory animal model experiments. The authors cite multiple references, including the U.S. Food and Drug Administration (FDA) guidelines, in an attempt to support their argument that drug dosages can be converted between species using a simple BSA conversion equation (3, 4). Although the paper has been cited >1000 times across a variety of disciplines over a 5-year span, investigation into the underlying science and interpretation of references behind the authors' arguments reveal major issues that severely limit the applicability of the recommendations and should cause major concern to researchers and clinicians.

Reagan-Shaw's argument appears to be rooted in sound discussion of one approach for selecting the safe starting dose for first-in-human clinical trials of cytotoxic agents but regularly extrapolates beyond the original intent of the sources it cites to make unfounded recommendations. The abstract and introduction of the work discuss the “safe starting dose for phase I studies of anticancer drugs from preclinical animal toxicology data” (3). As the paper continues, these recommendations inappropriately evolve into the argument that clinically effective therapeutic doses can be derived from animal model studies simply through a BSA conversion formula, with many references cited grossly out of context (4–8). This overly simplistic conversion neglects discussion of interspecies differences in drug absorbance, metabolism, clearance, *etc.* These differences in pharmacokinetics greatly affect the resulting peak plasma concentration (C_{max}) values and exposure derived from area under curve (AUC), which influence the dose-response relationship of potential therapeutics (1, 2). If scaling of an active dosage in experimental model species is applied as described by Reagan-Shaw *et al.* in human trials, it may have overtly negative consequences on drug development or jeopardize the safety of individuals participating in early clinical trials (3, 9). Through presenting the history of BSA scaling, reviewing the literature that

Abbreviations: AUC, area under curve; BSA, body surface area; C_{max} , peak plasma concentration; FDA, U.S. Food and Drug Administration; HED, human equivalent dose; LD₁₀, lethal dose for 10% of the sample; NOAEL, no observable adverse event level; PAD, pharmacologically active dose; PBPK, physiologically based pharmacokinetic modeling

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discredits this method, clarifying the FDA's recommendations on this issue, and using examples in which BSA scaling could have negative consequences, we aim to demonstrate why the recommendations presented by Reagan-Shaw *et al.* are inappropriate.

BRIEF HISTORY OF BSA SCALING

Reagan-Shaw's advocacy for the use of BSA for conversion of pharmacologically active doses (PADs) for therapeutic agents between species represents an apparent return to the body surface law from the 19th through the early 20th century, during which time it was common to claim that brain size, blood volume, heart size, or other biologic systems had a direct relation to BSA (10–13). The rationale of surface law is that the metabolism of all warm-blooded animals is directly related to their surface area, though this is likely partially due to poor techniques by modern standards in measuring surface area and basal metabolism (10, 11, 14, 15). For example, maintenance of $\sim 37^{\circ}\text{C}$ body temperature was accredited to a balance with the body surface for thermoregulation and was said to be a natural law obeyed by all animals (10, 12). Because the adherence to surface law was so prevalently accepted, it was even predicted that the individual cells of larger animals may be scaled according to the surface law (11). With advances in scientific measurement techniques and pharmacokinetic modeling, it is actually rare to find mention of the surface law in modern pharmacokinetic literature other than to teach a history of the field of pharmacokinetics (14, 15).

Reagan-Shaw *et al.* cite 2 notable works from the surface law paradigm of thought (16, 17). The first is Rubner's research from 1883 (16), which explored the metabolic rate of 7 dogs of varying sizes with the tools available at the time, devised a new method of measuring BSA, and then related this to basal metabolic rate (11, 12, 15). The second, a work by Pinkel in 1958 (17), suggests a seemingly closer relationship for a reported therapeutic dose of 5 chemotherapeutic drugs between humans and 1–3 of 4 selected species (rat, mouse, hamster, and 10 kg dog) when BSA scaling was used rather than by body weight scaling (17). Pinkel supports the claim that blood volume was scalable between species by BSA using works published by Dreyer and Ray in 1910 and in 1912 (18, 19), which were limited to comparisons among mice, guinea pigs, and rabbits (11, 17). Pinkel also cites work relating blood volume, plasma proteins, and glomerular filtration rate scaling by BSA between humans of various sizes, rather than between species. This claim is repeated by Reagan-Shaw *et al.*, such that, "correlations between blood volume, circulating plasma proteins, and renal function with BSA in several species also have been illustrated" (3), and cites Pinkel's weak and outdated paper (17) as sole reference for this statement.

The statements by Pinkel that blood volume, circulating plasma proteins, and renal function correlated with a surface law had already been disproven in the literature for over a decade when published in 1958, and there is no reason to perpetuate misinformation by allowing it to stay in the modern literature without proper historical context (10, 11, 15, 20). In 1947, Kleiber (11) made a *reductio ad absurdum* argument to discredit the claims of Dreyer and Ray (18, 19), which is equally applicable to Pinkel (17)

from 1958 and Reagan-Shaw *et al.* (3) from 2008. Kleiber argued that if blood volume is proportional to BSA, and a man is 5% blood by mass, then a rat would be $\sim 40\%$ blood by mass (11). In reality, the reported blood volume of the mouse is 58–79 ml/kg for its size of 20–63 g, and the human has a total blood volume of ~ 65 –82 ml/kg per average adult (21–23), such that both species are ~ 5 –8% blood by weight. Measures of plasma proteins are also similar among mice, rats, and humans of ~ 60 g/L with 40–50% being albumin (24–26). The volume of plasma and concentration of plasma proteins actually scale more closely to lean body mass, and therefore, plasma volume alone does not account for differences in metabolism and pharmacokinetic values for the 5 drugs explored by Pinkel correlating to BSA (5, 12, 14, 17). The claim of kidney function scaling by BSA greatly understates the complexity of the kidney function between species (27). Rodents have a variety of adaptations for different environments independent of BSA or size, specifically relating to urine-concentrating ability for those in arid environments (28). In studies between human patients, it has been found that lean body mass is the strongest correlating factor to kidney function (5). The observation that clearance of certain small molecules is more rapid in the mouse and rat is dependent on the rate of circulation, where renal and hepatic blood flow is nearly 4 times higher than in human (29, 30).

MISINTERPRETATION OF RECOMMENDATIONS FOR BSA DOSE CONVERSION

Reagan-Shaw *et al.* state that "...the Food and Drug Administration has suggested that the extrapolation of animal dose to human dose is correctly performed only through normalization to BSA..." (3). They continue to describe interspecies conversion of the dosage used to achieve therapeutic benefit in animal studies into human clinical trials and provide an example using the small molecule resveratrol (which will be addressed separately). It is erroneous to claim that the FDA guidelines state that BSA properly converts dosages from animal trials into a dosage in humans that elicits therapeutic effect. In the glossary of the FDA guidelines cited by Reagan-Shaw *et al.*, the FDA clearly distinguishes between definitions for human equivalent dose (HED; used multiple times in Reagan-Shaw) and PAD (4). According to the FDA guidelines, the definition of HED is solely for establishing a safe starting point for first-in-human trials, based on the no observable adverse event level (NOAEL) or the no observable event level in animal models (4). Thus, it must be emphasized that, according to the FDA, conversion of a drug dosage from model species to humans by the HED is not inherently expected to elicit a physiologic effect or PAD (4). This is further clarified in the updated version of the FDA guidelines for safe starting dosages in humans from 2005 (29). It is clear that HED and PAD are 2 distinct dosages that are not interchangeable, and thus, BSA conversion is only applicable to HED. The FDA does not recommend a method to derive a PAD from model species, nor does the FDA state that scaling by BSA is the "only" method to scale for first-in-human trials (4, 31).

Although the BSA conversion equation is accepted for one purpose in pharmacology (*i.e.*, as a potential scaling

method for selecting a safe starting dosage), it has been inappropriately used to justify the clinical potential for multiple investigational therapeutics undergoing animal trials from this misinterpretation of the FDA recommendations by Reagan-Shaw *et al.* These examples include protease inhibitors for hepatitis C (32), serotonin reuptake inhibitor for Alzheimer's disease (33), nuclear transport modifier peptide to treat inflammation (34), and antibody-mediated delivery of IL-2 for the treatment of breast cancer (33). If the misinterpretation that human trials can proceed simply by scaling dosage according to BSA were to somehow move forward unchecked, it could lead to inefficacy due to underdosing or dangerous outcomes for first-in-human volunteers due to poor clearance or unforeseen reaction to biologic therapeutics due to interspecies differences in physiology and toxicology (1, 4, 31, 36). According to Marchetti and Schellens [2007 (37)], "40% of exits from Phase I trials are caused by inappropriate pharmacokinetics of the test compound." An admittedly extreme example would be the case of underestimating toxicity in a first-in-human trial, as in the case of the investigational antibody TGN1412 (36). An intravenous dosage 1/500 of the recommended safe dosage derived from rodents placed 6 volunteers in the intensive care unit for multiorgan failure requiring 8–16 days of life support (36, 38). Simply converting a safe dosage by BSA for Reagan-Shaw's "human equivalent dosage," or administering 1/12 of what is considered safe in rodents, may have killed these 6 first-in-human volunteers (36, 38). This is in direct contrast to the unsupported statement by Reagan-Shaw *et al.* that "BSA normalization of doses must be used to determine safe starting doses of new drugs because initial studies conducted in humans, by definition, lack formal allometric comparison of the pharmacokinetics of absorption, distribution, and elimination parameters" (3). Likewise, the AUC of deramciclane in humans was approximately an order of magnitude higher than would have been predicted by BSA (1, 39). In contrast, the injectable antisense synthetic oligonucleotide, now marketed as Kynamro (Genzyme Corporation, Cambridge, MA, USA) for dyslipidemia, showed desired pharmacokinetics when scaled by milligram per kilogram between model species and man (40).

In describing the utility of BSA in clinical medicine, Reagan-Shaw *et al.* cite many works that focus on the use of BSA scaling between human patients of different sizes, with an emphasis on cancer therapeutics. As such, their discussion of BSA scaling to adjust a starting dose to an individual patient is not relevant to BSA scaling to translate an animal dosage into a human dosage. A thorough critique of the limitations of BSA scaling for adjusting drug dosages between individual patients is beyond the scope of this paper, but it is important to recognize that even this clinical application is questionable, and the references used by Reagan-Shaw reveal considerable opposition to it. For instance, Sawyer and Ratain [2001(5)] conclude that BSA scaling "has spread throughout the practice of oncology with little justification," and "very few of the organ functions that determine pharmacokinetics of a drug are related to body surface area." Similarly, Baker *et al.* (7) acknowledge that BSA is not used to establish a therapeutic dosage and state that "although the primary objective of phase I trials is to evaluate drug toxicity, antitumor activity

is usually a secondary objective." Likewise, Kaestner and Sewell (8) concede that BSA scaling may be used for starting doses in man but conclude that "the gradual discontinuation of BSA-based dosing of cytotoxic drugs in clinical practice is seemingly justified." According to Gurney (6), BSA adjustments frequently lead to underdosing, and "the inaccuracy of body surface area-dosing is more than an inconvenience and it is important that methods for more accurate dose calculation are determined." Again, it must be emphasized that clinical use of BSA to scale drug dosage between human patients of different sizes is not universally accepted due to its many flaws. Interindividual scaling represents a completely different application than deriving therapeutic dosage from animal models, where drug absorption, metabolism, clearance, and toxicology may differ considerably from humans.

ACCEPTED METHODS FOR ESTIMATING PHARMACOKINETIC PARAMETERS

The literature shows that it is not acceptable to estimate a safe and pharmacologically relevant dosage of a therapeutic in humans by blindly scaling the milligram per kilogram dosage (*i.e.*, body mass) from rodent studies, yet it is clear that BSA is also not appropriate (1, 2, 30, 38, 41). Correlations of PAD with BSA for specific drugs are simply correlative, have been overstated, and the use of BSA in clinical practice is not supported by pharmacokinetic data (5, 8, 17, 42). The use of BSA for prediction of interspecies drug toxicity reemerged with Schein *et al.* [1970 (43)], which was cited by Reagan-Shaw. This work has been discussed by Dixon [1976 (42)] who showed that expressing dosages with BSA or body mass were similar in their inaccuracies between larger species for calculating toxicity in first-in-human clinical trials (42, 43). The body structures and function of mammals do not simply scale by BSA or body mass, with many factors affecting the outcome of a drug dosage beyond differences in size (9, 27). Furthermore, the oral absorption of small molecules can be extremely variable between rodents and humans due to interspecies differences in organ structure and drug-metabolizing enzymes in the small intestines (44). Ultimately, the goal is to find a dosage that will allow for therapeutic concentrations of the small molecule to the cellular target without toxicity (1). With the high cost of drug discovery in time and resources, there are commonly used methods available to estimate these parameters for a human dosage of drugs from animal studies to pick promising drug candidates (14). All aspects of pharmacokinetics including absorption, distribution, metabolism, excretion, and toxicity of the drug are summarily taken into consideration to estimate resulting plasma levels and exposure from a dosage of a potential therapeutic compound (1, 2, 45). A detailed discussion of each method of animal-to-human dosage prediction is beyond the scope of this paper, and detailed resources are available elsewhere (46). As such, we provide a very brief summary of currently accepted methodologies for estimating interspecies pharmacokinetic parameters, and the pharmacologically relevant dosage of a therapeutic agent during early exploration.

The use of allometry for pharmacokinetic prediction originates from work in metabolic scaling, which plotted the formula to fit the metabolic rate and body weight of animals on double logarithmic scale (10, 29, 41). Although Reagan-Shaw *et al.* repeatedly refer to BSA conversion as allometry, it is actually the practice of isometry due to direct scaling of proportions (3, 12, 14). The allometric approach for prediction of pharmacokinetic parameters requires pharmacokinetic data availability for at least 2 model species and is prone to high errors but is straightforward for a broad scientific audience, inexpensive, and has been more accurate than BSA scaling for small molecules (14, 29). Despite its

high error rate, the high cost of human trials makes this low-cost method practical for general prediction when multiple animal studies are available (14, 29, 41). Allometry has various modifications and corrective factors for prediction that can be used to quickly approximate the range of human pharmacokinetic parameters, such as circulation time, clearance, or blood flow (29, 41). The literature has examples where allometric conversions do hold true, yet it remains a purely correlative method (2, 47). Scaling by allometry is not based on physiology and does not consider interspecies differences contributing to the high error rate and multiple pitfalls (14, 41).

With the costs and complexity of drug development, the scientific community has a deep interest in exploring and modeling the differences between model species and humans (45, 48, 49). Compartmental models in pharmacokinetic prediction were first used in the 1930s and have improved through an understanding of physiology and pharmacology (30, 48, 49). Modern physiologically based pharmacokinetic modeling (PBPK), though mathematically complex, is becoming more practical through commercial computer programs (2, 49, 50). This method describes the body as multiple compartments that models individual organs and tissues within organs, to the detail of estimating the volume of intracellular spaces (2, 30, 45, 49). The physical properties of the therapeutic agent and its likely species-specific metabolism are also considered (30, 45). From *in vivo* data on the small molecule of interest, and reported physiologic differences between species, the base mathematical model can be adjusted to predict absorption, volume of distribution, exposure, and clearance of a potential therapeutic from early development phases (30, 45, 50). An important value to PBPK is mentioned by Parrott *et al.* (50), where "...the method is also able to include estimates of variability and uncertainty in the predictions." The authors recommend Atkinson and Smith (48) for an overview of PBPK, Aldo Rescigno's 2010 historical review of compartmental modeling with a guided example (49), or Gerlowski and Jain's discussion on compartmental modeling for PBPK (30).

REVISITING THE RESVERATROL EXAMPLE

Reagan-Shaw's example for the "inappropriate comparisons between studies" for pharmacologic effect in humans stemmed from the discussion of resveratrol dosage in the literature and press (3, 51, 52). The authors use BSA scaling to state that the positive physiologic effects of 22.4 mg/kg resveratrol in chow provided to genetically homogeneous mice should scale to a 1.82 mg/kg dosage in humans (3, 51). This method actually contradicts what the authors state themselves: "For the purpose of initial clinical trials in healthy adult volunteers, the HED is calculated using BSA normalization of the animal dose where no observed adverse effects were observed" (3). Thus, the HED is meant to be calculated only using one very specific dosage—that at which NOAEL occurs. In the case of resveratrol, the conservative estimate of NOAEL of 200 mg/kg is obtained from rats (51, 52). The BSA equation provided in the text yields an HED of 32.4 mg/kg for humans, which is 1920 mg in a 60 kg adult. If the 600 mg/kg NOAEL derived in dogs is used instead, the safe starting dose in humans would be an astounding 324 mg/kg, or ~19,000 mg for a 60 kg adult. An alternative method to determine a safe starting dose is the FDA's recommendation of 1/10 of the BSA-scaled lethal dose for 10% of the sample (LD₁₀), also specified by Reagan-Shaw (54). Using the data from Crowell *et al.* (53), in which 3000 mg/kg can be considered the LD₁₀ in healthy male rats, a safe starting dose can be calculated to

be 48.7 mg/kg, or ~2900 mg/d in a 60 kg adult. Regardless of these figures, the purpose of the HED is to provide a conservative estimate for a safe starting dose for first-in-human clinical trials, and it is not appropriate to use the BSA conversion equation to translate from other arbitrary dosages as an indicator of PAD. In other words, it is not appropriate to use the BSA conversion formula, or any generic formula for that matter, to translate the 22.4 mg/kg dosage of resveratrol used by Baur *et al.* (51), or any animal model study, into a human equivalent dosage for therapeutic effect. Furthermore, because the equation was originally meant for cytotoxic drugs, the use of BSA scaling is not appropriate for resveratrol, given that it is not cytotoxic.

Another concern with the "human equivalent dose" of resveratrol derived by Reagan-Shaw *et al.* is that it is presented without discussion of the reported C_{max} or AUC to estimate tissue distribution and activity between species (1–3). These are basic pharmacologic factors for estimating the potential of a small molecule to affect its cellular target *in vivo* (1, 2). For instance, Asensi *et al.* [2002 (55)] reported that a single dosage of 20 mg/kg in mice yields a C_{max} of 592 ng/ml. When the 20 mg/kg mouse dosage is scaled according to the interspecies BSA conversion, the "human equivalent" is 1.62 mg/kg, or ~98 mg for a 60 kg human (3, 55). If pharmacokinetic parameters scale to BSA, a dosage of 100 mg should yield a comparable C_{max} and/or AUC to those seen in the mouse trial (17). Yet, a single human dosage of 100 mg was investigated by Almeida *et al.* [2009 (56)], which resulted in a C_{max} of 21.4 ng/ml, ~1/27 of that of an "equivalent" mouse dosage. When 4 hours exposure is calculated by the trapezoidal method, the BSA-scaled 1.62 mg/kg dosage of resveratrol has an AUC_{0–240min} of 19.5 ng × h × ml⁻¹ in humans; only 1/3 of that was achieved using a supposedly equivalent 20 mg/kg in mice in only 1 h (AUC_{0–60min} = 63.4 ng × h × ml⁻¹) (55, 56). It is clear that species-specific differences in absorption and metabolism of resveratrol have a significant influence on pharmacologic parameters, and it should not be surprising that BSA scaling is not accurate for dosage conversions. Multiple works have reported safety and pharmacokinetic parameters of resveratrol in humans (57–60) and rodents (55, 61–64), and therefore, human clinical trials involving resveratrol are no longer "first-in-human" studies and were not at the time of Reagan-Shaw's publication.

CONCLUSIONS

The 2008 paper by Reagan-Shaw *et al.* (3) has been extensively used to justify BSA conversion as a method to translate the pharmacologically active dosage of drugs from animals to humans but lacks scientific support. Although scaling by BSA is still currently used to estimate a safe starting point in first-in-human clinical trials of cytotoxic drugs when the NOAEL or LD₁₀ in animal models is known, and is also clinically used for determining patient-specific dosages of cancer chemotherapy drugs, the use of BSA scaling is based on antiquated ideas that have been discredited and therefore is rarely justified in modern science. Contrary to the paper's claim, the FDA does not advocate BSA or any specific method for

determining therapeutic dosage range in humans. Many of the citations of Reagan-Shaw *et al.* are flawed or are not applicable to interspecies scaling and ultimately do not support the claims of the authors. It is necessary for researchers working in translational medicine to recognize the fatal flaws of using BSA conversions to estimate PAD in humans, so that future human clinical trials do not yield spuriously negative findings based on major underestimations for therapeutic dose, or severe adverse events resulting from oversimplification of dose conversion. After safety is established, appropriate selection of the PAD for human clinical trials should consider other validated approaches that use physiologic and pharmacokinetic data from animal models and humans to more accurately estimate therapeutic range. **FJ**

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