A Single-Species Approach Considering Additional Physiological Information for Prediction of Hepatic Clearance of Glycoprotein Derivate Therapeutics

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Abstract

Background and Objectives: Existing methods for the prediction of human clearance of therapeutic proteins involve the use of allometry approaches. In general, these approaches have concentrated on the role of body weight, with only occasional attention given to more specific physiological parameters. The objective of this study was to develop a mechanism-based model of hepatic clearance (CL_H), which combines a single-species scaling approach with liver physiology, for predicting CL_H of selected glycoprotein derivate therapeutics, and to compare the outcome of this novel method with those of two empirical methods obtained from the literature – namely, the single-exponent theory and multiple-species allometry. Thus, this study was designed as an explanatory study to verify if the addition of physiological information is of benefit for extrapolating clearance of selected therapeutic proteins from one species to another.

Methods: Five glycoprotein derivate therapeutics that are known to be principally eliminated by asialoglycoprotein receptors (ASGPRs) under *in vivo* conditions were selected. It was assumed that the interspecies differences in CL_H reported for these compounds are reflected by the interspecies differences in the abundance of these receptors. Therefore, key scaling factors related to these differences were integrated into one model. Fourteen extrapolation (prediction) scenarios across species were used in this study while comparing the single-species model, based on physiology, with the single-exponent theory. In addition, the physiological model was compared with multiple-species allometry for three proteins.

Results: In general, the novel physiological model is superior to the derived allometric methods. Overall, the physiological model produced a predicted CL_H value with levels of accuracy of 100% within 3-fold, 100% within 2-fold and about 82% within 1.5-fold, compared with the observed values, whereas the levels of accuracy decreased to 93%, 77% and 53%, respectively, for allometry. The proposed physiological model is also superior to allometry on the basis of the root mean square error and absolute average fold error values.

Conclusions: It has been demonstrated that interspecies differences in the abundance of ASGPRs principally govern interspecies variations in CL_H of compounds that are principally eliminated by ASGPRs. Overall, the proposed physiological model is an additional tool, which should facilitate investigation and prediction of human CL_H of specific glycoproteins solely on the basis of clearance data determined in a single preclinical species.

Background

Early prediction of human pharmacokinetics of therapeutic proteins is playing an increasingly important role in drug development. A review of protein clearance has shown that excellent allometric relationships (clearance versus body weight) can exist between animals and humans.[1-8] Some authors have recently revisited the allometric scaling principles of clearance of 34 therapeutic proteins, and found that the optimal exponent was estimated to be about 0.8.[6] Recently, different methods for projecting human clearance from animal pharmacokinetic data were examined for 13 therapeutic monoclonal antibodies exhibiting linear pharmacokinetics over the tested dose ranges.[7] A better correlation between the observed human clearance and the estimated human clearance was obtained by using pharmacokinetic data from cynomolgus monkeys, with an optimal

scaling exponent of 0.85, than by using other scaling approaches. Therefore, the single species-scaling approach using a fixed exponent (the single-exponent theory) affords a viable alternative to predict human plasma hepatic clearance (CL_H) of several therapeutic proteins, especially in the absence of non-linear pharmacokinetics and species-specific clearance mechanisms.[1-7] Conversely, in studying the allometric scaling of clearance of coagulation factors and tissue-type plasminogen activators, Mahmood[8] observed a wider range of exponents (0.67–0.91), indicating that the use of a fixed-exponent approach could be inappropriate in other cases. Therefore, Mahmood[8] demonstrated that single-species scaling could be useful, but the predicted clearance may not be as accurate as that predicted from scaling of three or more species. If, for some unforeseen reason, one has to use the single-exponent theory (about 0.8) or multiple-species allometry, then extreme caution is needed in the interpretation of data, as the predicted clearance may be in error.[8] Therefore, there is still scope to challenge these two empirical approaches (the single-exponent theory and multiple-species allometry).

Accumulation of information on the role of physiology in the extrapolation procedure should be encouraged. Standard allometry is normally based on the body weight, which limits the investigation of specific physiological determinants that could also influence the prediction of clearance. Alternative approaches to the use of a body weight value for allometry should include those that are more closely based on liver physiology, since this organ expresses several receptors that are involved in CL_H of several therapeutic proteins under *in vivo* conditions. Therefore, the involvement of this aspect in the prediction of CL_H of therapeutic proteins has been considered likely. The clearances of high-molecular-weight compounds from plasma are usually due to cellular uptake by endocytosis, especially in the liver and the kidneys, and/or to irreversible binding of these compounds to plasma proteins. Numerous studies have suggested that low-density lipoprotein receptor-related proteins (LRPs), asialoglycoprotein receptors (ASGPRs) and mannose receptors (MRs) present on the liver cell surface might play important roles as clearance mechanisms for several therapeutic proteins under *in vivo* conditions.[9-18] In particular, high-mannose oligosaccharides have clearance governed by MRs, whereas LRPs – an oligosaccharide-independent mechanism – are members of the low-density lipoprotein (LDL) receptor family, which also play a role in endocytosis. ASGPRs depend on removal pathways that involve glycan recognition. Hepatic ASGPRs are lectins, which bind glycoproteins from which a sialic acid has been removed to expose terminal galactose residues. These receptors remove the target glycoproteins from the circulation.[12-18] Because of its specificity, predominant expression on hepatocytes and high capacity for receptor-mediated endocytosis, the ASGPR has been validated as a target for CL_H of several therapeutic proteins such as erythropoietin, tissue plasminogen activators, interferon and various hormones.[3,8,19-27]

Literature data suggest that the amino acid composition of ASGPRs is closely related between species. For example, there is 80% amino acid homology between the major human (H1) and rat (RHL1) ASGPR subunits.[26] In addition, a review of protein clearance has shown that excellent allometric relationships can exist between animals and humans for substrates of ASGPRs.[1] These observations should indicate conservation of the function/affinity of the clearance mechanism across species. Therefore, it could be assumed that the variation in the affinity between compounds in a single species is greater than the variation in the affinity between species for a single compound. The mechanism of CL_H must be assessed prior to any scaling. In this case, if the primary receptors that are related to the hepatic uptake of a therapeutic protein are related to ASGPRs, it could consequently also be assumed that the interspecies differences in clearance reported for a glycoprotein that is eliminated by ASGPRs are reflected by the interspecies differences in the abundance of these receptors. In this context, the number of ASGPRs per gram of liver (or per kilogram of body weight) in several preclinical species could easily be estimated from the literature.[13-18] Moreover, significant interspecies differences in the abundance of ASGPRs have been reported. Whether or not this may explain the interspecies differences in clearance is still not known for compounds that are mainly eliminated by ASGPRs. Therefore, such a novel principle of scaling should also be challenged.

The objective of this study was to develop a mechanism-based model of CL_H, which combines a single-species scaling approach with liver physiology, for predicting CL_H of selected glycoprotein derivate therapeutics that are principally eliminated by ASGPRs, and to compare the outcome of this novel method with those of two methods obtained from the literature – namely, the single-exponent theory and multiple-species allometry. Thus, this study was designed as an explanatory study to verify if the addition of physiological information is of benefit for extrapolating clearance of selected therapeutic proteins from one species to another.

Methods

This study was performed in two steps. The first step consisted of development of the single-species model of CL_H, based on liver physiology, and the second step consisted of comparison

of this novel model with more empirical models that have been reported in the literature (namely, the single-exponent theory and multiple-species allometry).

The Dataset

Compounds were considered only if their *in vivo* CL_H is principally mediated by ASGPRs (according to mechanistic experimental studies published in the literature) and if the necessary preclinical and clinical data were available. In other words, if the primary receptors related to the hepatic uptake of a carbohydrate protein seemed to be a different type of receptor (e.g. LRPs, MRs), that protein was not further analysed in this study. Therefore, the contribution of LRPs and MRs should be small. Five glycoprotein derivate therapeutics that are known to be principally eliminated by ASGPRs under *in vivo* conditions were selected on the basis of a review of the literature.[3,8,19-27] Recombinant human erythropoietin (rhEPO) is a glycoprotein hormone, which controls erythropoiesis, or red blood cell production. It is a cytokine for erythrocyte precursors in the bone marrow.[3,19,20] Lanoteplase, reteplase (BM 06022) and pamiteplase are recombinant plasminogen activators which, when administered as a single-bolus intravenous injection, display thrombolytic activity.[8,21-25] Finally, recombinant neutrophil inhibitory factor (rNIF) [UK 279276] is a glycoprotein which has also been investigated as an intravenous treatment for stroke.[26,27] Since the pharmacokinetics of rNIF can be non-linear in dogs,[27] the clearance value studied at the lowest dose was considered for this compound. These proteins have clearance data in more than one preclinical species and in humans. The corresponding average value of clearance determined *in vivo* is presented for each species and compound in table I.

Modelling Assumptions

A non-saturable, high-affinity/high-capacity clearance process is assumed to be present in all animal species, as well as in humans, for each compound studied, and it is believed to involve hepatic uptake mediated by ASGPRs. Consequently, it was considered that the sole factor governing the interspecies variation in CL_H under *in vivo* conditions is the interspecies difference in the content of ASGPRs, as previously assumed.

Development of the Physiological Model

Physiological Modelling of Hepatic Clearance (CL_H)

Development of the physiological model of CL_H was performed by combining the value of clearance observed in one

Table I. Input parameters reported for five therapeutic proteins

Therapeutic protein	Species	Observed CL (mL/min/kg)[a]	BW (kg)[b]
rhEPO	Mouse	1.189	0.025
	Rat	0.230	0.30
	Dog	0.157	10
	Monkey	0.206	3.2
	Human	0.135	68
Lanoteplase	Rat	1.10	0.215
	Human	0.561	70
Reteplase (BM 06022)	Rat	7.6	0.495
	Dog	5.0	30
	Monkey	5.3	9.4
	Human	4.3	70
Pamiteplase	Rat	5.28	0.125
	Dog	2.44	11
	Monkey	1.17	5
	Human	2.2	65
rNIF (UK 279276)	Rat	0.092	0.25
	Dog	0.06	12

a Average values were obtained from the literature.[3,8,19-27] Clearance refers to plasma kinetics and it was assumed that this is due to CL_H.

b According to the literature sources.

BW = body weight; **CL** = total body clearance; **CL_H** = hepatic clearance; **rhEPO** = recombinant human erythropoietin; **rNIF** = recombinant neutrophil inhibitory factor.

species with key scaling factors obtained from the literature, which are related to interspecies differences in the abundance of ASGPRs. Since the initial literature data expressed the abundance of ASGPRs in terms of total receptors per cell of liver, extrapolation factors were essential to convert the total receptors per cell of liver to total receptors per gram of liver, and subsequently to total receptors per kilogram of body weight (equations 1 and 2):

$$CL_{\text{target species}} = in\ vivo\ CL_{\text{index species}} \times ISF_{\text{target species / index species}} \quad \text{(Eq. 1)}$$

$$ISF_{\text{target species / index species}} = (AF \times HF \times LF)_{\text{target species / index species}} \quad \text{(Eq. 2)}$$

where CL is the *in vivo* clearance referring to plasma kinetics (either observed or predicted, measured in mL/min/kg) in animals and humans; $ISF_{\text{target species/index species}}$ is the global interspecies scaling factor (i.e. total receptors per unit kilogram of body weight in the target species relative to the index species); AF is the specific factor for abundance, which corresponds to

physiological differences in the abundance of ASGPRs in the liver (i.e. total receptors per cell of liver in the target species relative to the index species); HF is the specific factor for hepatocellularity, which corresponds to physiological differences in cellularity (i.e. the number of cells per gram of liver in the target species relative to the index species); and LF is the specific factor considering the differences in the fractional content of the liver (i.e. grams of liver per kilogram of body weight in the target species relative to the index species). Table II presents details of the ISF values resulting from each species that was studied, whereas table III presents the global ISF$_{target\ species/index\ species}$ values according to the extrapolation scenarios in figure 1.

Estimation of the Abundance Factor

The total number of ASGPRs per cell of liver in each species was obtained from experimental studies published in the literature.[13-18] Briefly, *in vitro* binding studies of a ligand of ASGPR, asialofetuin, were performed at 37°C with animal and human hepatocytes to estimate the numbers of these receptors.[13-18] These studies gave the numbers of cell surface occupied receptors, assuming that one asialofetuin molecule binds to one receptor molecule. Using fresh hepatocytes, the total numbers of ASGPRs were estimated to be 2.44×10^5 receptors/cell in mice, 5.21×10^4 receptors/cell in dogs and 1.4×10^5 receptors/cell in humans.[13-18] For rats, there is more than one value reported in the literature (1.8×10^5, 2.1×10^5 and 4.08×10^5 receptors/cell). For the purpose of this study, the intermediate value was used (2.1×10^5 receptors/cell) [table II].

Table II. Calculation of scaling factors for each species

Species	Natural values for each species			
	AF (receptor) [ASGPRs/liver cell][a]	HF (cellularity) [10^6 cells/g liver][b]	LF (weight) [g liver/kg BW][c]	ISF (AF × HF × LF) [ASGPRs/kg BW]
Mouse	2.44×10^5	135	88	2.88×10^9
Rat	2.10×10^5	117	37	8.97×10^8
Dog	5.21×10^4	215	32	3.58×10^8
Human	1.40×10^5	100	26	3.60×10^8

a   Average values were obtained from the literature.[13-18] Fresh hepatocytes were used.

b   Average values were obtained from the literature.[28,29]

c   Average values were obtained from the literature.[30]

AF = abundance factor; **ASGPR** = asialoglycoprotein receptor; **BW** = body weight; **HF** = hepatocellularity factor; **ISF** = interspecies scaling factor; **LF** = liver factor.

Table III. ISF$_{target\ species/index\ species}$ values used in the single-species model based on physiology[a]

Global ISF	Value
ISF$_{rat/mouse}$	0.31
ISF$_{dog/mouse}$	0.12
ISF$_{human/mouse}$	0.12
ISF$_{dog/rat}$	0.40
ISF$_{human/rat}$	0.40
ISF$_{human/dog}$	1.0

a   ISF ratio between the target species and the index species, as described in the Methods section, according to the extrapolation scenarios shown in figure 1. The natural ISF value for each species was obtained from table II.

ISF = interspecies scaling factor.

Estimation of the Hepatocellularity Factor

Hepatocellularity, expressed as 10^6 cells per gram of liver, is assumed to be 135, 117, 215 and 100 in mice, rats, dogs and humans, respectively (table II).[28,29] In humans, this value ranged from 74 to 139.[28,29] Again, the intermediate value (100) was used in this study.

Estimation of the Liver Factor

The fractional content of the liver (grams of liver per kilogram of body weight) is equal to 88, 37, 32 and 26 in mice, rats, dogs and humans, respectively (table II).[30]

Allometric Modelling of CL$_H$

Single-Species Extrapolation Based on the Single-Exponent Theory

Single-species-based allometry (the single-exponent theory) has been reported in several studies.[6-8] Equation 3 shows this approach:

$$CL_{target\ species} = CL_{index\ species} \times \left(\frac{BW_{target\ species}}{BW_{index\ species}}\right)^{0.8} \quad \text{(Eq. 3)}$$

where CL referring to plasma kinetics is expressed as mL/min and body weight (BW) is expressed in kilograms.

Multiple-Species Extrapolation

For multiple-species allometry, a power function was applied (equation 4):

$$y[CL_{human}] = b(x[BW])^m \quad \text{(Eq. 4)}$$

Using log-log transformed data, equation 4 transforms into a linear function (equation 5):[1]

$$\log(y[CL_{human}]) = m\log(x[BW]) + \log(b) \quad \text{(Eq. 5)}$$

where m is the slope and log(b) is the intercept resulting from the correlations between CL referring to plasma kinetics

(mL/min) [y axis] and BW (kilograms) [x axis] that have been observed in the preclinical species.

Comparative Assessment Between the Physiological Model and Allometry

Single-Species Model Based on Physiology versus the Single-Exponent Theory

Investigated Extrapolation Scenarios

Fourteen extrapolation (prediction) scenarios across species were used in this study for five therapeutic proteins, to assess the single-species models of CL_H (this study and the single-exponent theory), as depicted in figure 1.

Single-Species Model Based on Physiology versus Multiple-Species Allometry

A complete preclinical and clinical dataset was available for three proteins: rhEPO, reteplase and pamiteplase (table I). Consequently, multiple-species allometry was applied to these compounds and compared with the proposed physiological model for prediction of human CL_H.

Input Parameters

The input data used in the studied models are shown in tables I–III.
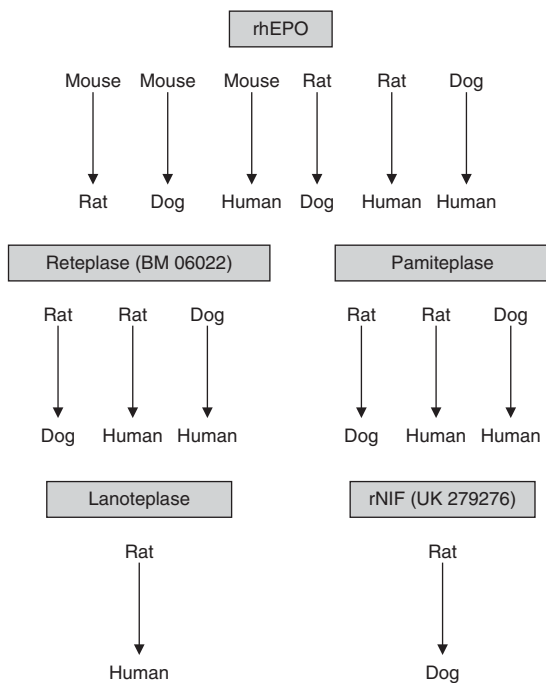

Fig. 1. Scenarios investigated in this study to assess the single-species scaling approaches used for prediction of CL_H *in vivo* (from the index species to the target species). CL_H = hepatic clearance; **rhEPO** = recombinant human erythropoietin; **rNIF** = recombinant neutrophil inhibitory factor.

Statistical Analyses

The prediction accuracy was assessed by comparing predicted and observed values of CL_H, using specific statistical parameters.[31] Specific fold errors of deviation between the predicted and observed values (% predictions within fold errors of ≤1.5, ≤2 and ≤3) were calculated, as well as the coefficient of correlation (r) values. Furthermore, absolute average fold errors (AAFEs) and root mean square errors (RMSEs) were used as a measure of spread and to rank precision, respectively.[31] Finally, plots of predicted versus observed CL_H values (mL/min/kg) were also created.

Results

Comparative Assessment Between the Physiological Model and Allometry

Single-Species Model Based on Physiology versus the Single-Exponent Theory

In most cases, the novel physiological model was superior to allometry derived from the single-exponent theory for the 14 extrapolation scenarios that were investigated for five therapeutic proteins. In this study, the physiological model predicted CL_H with levels of accuracy of 100% within 3-fold, 100% within 2-fold and about 79% within 1.5-fold, compared with the observed value, whereas the levels of accuracy decreased to 93%, 86% and 57%, respectively, for allometry. The proposed physiological model was also superior to allometry on the basis of the RMSE and AAFE values (table IV). Both approaches resulted in a high degree of correlation between the predicted and experimental values, but with a slightly higher correlation for the single-exponent theory (r = 0.97) than for the physiological model (r = 0.94). This was also reflected graphically (figure 2). Note that use of an exponent of 0.75 in equation 3 (the single-exponent theory) compared with 0.8 (in this study) did not provide a superior level of accuracy, in general (data not shown).

An additional finding was that the scaling of CL_H *in vivo* was relatively proportional to the interspecies differences in liver abundance of ASGPRs (the ISF). In general, the greater the number of ASGPRs in a species (ISF values in table II), the greater the CL_H (per kilogram of body weight) and vice versa (table I). For example, CL_H *in vivo* for humans and dogs was nearly the same (per kilogram of body weight) for all compounds for which these data were available (rhEPO, reteplase and pamiteplase) [table I]. The ratio of the ISF (per kilogram of body weight) between these two species was unity (table III), which means that the scaling factor was unity.

Table IV. Comparative assessment between the single-species models

Parameter	Extrapolation scenario[a]	Predicted CL_H (mL/min/kg)			Observed CL_H (mL/min/kg)[d]	Fold error[e]	
		target species	physiological model[b]	single-exponent theory[c]		physiological model	single-exponent theory
Therapeutic proteins							
rhEPO	Mouse to rat	Rat	0.369	0.72	0.230	1.6	3.13
	Mouse to dog	Dog	0.143	0.36	0.157	0.91	2.29
	Rat to dog	Dog	0.092	0.11	0.157	0.59	0.70
	Mouse to human	Human	0.143	0.24	0.135	1.06	1.78
	Rat to human	Human	0.092	0.077	0.135	0.68	0.57
	Dog to human	Human	0.157	0.11	0.135	1.16	0.81
Lanoteplase	Rat to human	Human	0.44	0.35	0.561	0.78	0.62
Reteplase	Rat to dog	Dog	3.04	3.35	5.0	0.61	0.67
(BM 06022)	Rat to human	Human	3.05	2.82	4.3	0.71	0.65
	Dog to human	Human	5.02	4.22	4.3	1.17	0.98
Pamiteplase	Rat to dog	Dog	2.11	2.16	2.44	0.86	0.89
	Rat to human	Human	2.12	1.49	2.2	0.96	0.68
	Dog to human	Human	2.45	1.69	2.2	1.11	0.77
rNIF (UK 279276)	Rat to dog	Dog	0.04	0.04	0.06	0.67	0.67
Statistics							
≤1.5-fold error (%)						79	57
≤2-fold error (%)						100	86
≤3-fold error (%)						100	93
Correlation coefficient (r)						0.94	0.97
RMSE						0.13	0.23
AAFE						1.30	1.55

a   As depicted in figure 1.

b   Calculated using equations 1 and 2, developed in the present study. The physiological ISFs were obtained from table III. The predicted CL_H corresponds to the input CL_H data × ISF, as described in the Methods section. The input parameters on CL_H were obtained from table I.

c   Calculated using equation 3. The input parameters on CL_H and BW were obtained from table I.

d   Obtained from table I.

e   Fold error of the difference between the predicted and experimental values.

AAFE = absolute average fold error; **BW** = body weight; **CL_H** = hepatic clearance; **ISF** = interspecies scaling factor; **rhEPO** = recombinant human erythropoietin; **RMSE** = root mean squared error; **rNIF** = recombinant neutrophil inhibitory factor.

Single-Species Model Based on Physiology versus Multiple-Species Allometry

Multiple-species allometry provided a high degree of correlation and exponent values ranging from 0.60 to 0.89. With allometry, CL_H was underpredicted in comparison with the observed CL_H by about a 2-fold error for two compounds out of three (rhEPO and pamiteplase) [table V]. For the other compound (reteplase), CL_H was adequately predicted. Again, the proposed physiological model is superior to multiple-species allometry because CL_H was more accurately predicted

for two out of the three compounds that were studied (rhEPO and pamiteplase). For reteplase, the two approaches provided a similar level of accuracy (table V).

Discussion

The present study had the merits of (i) proposing a novel physiological approach for the prediction of CL_H of therapeutic proteins; (ii) exploring the validity of this approach by using five therapeutic proteins and, in particular, several

extrapolation scenarios across diverse species (14 scenarios); (iii) comparing the physiological approach with standard allometry for the current dataset of proteins; (iv) offering a first attempt to explore further mechanisms, by associating interspecies differences in the abundance of ASGPRs with interspecies differences in CL_H values; (v) accumulating information on the role of liver physiology in the extrapolation procedure; and (vi) suggesting ways of applying this approach in drug development. These aspects are discussed further below.

The main route of elimination greatly influences the predictability of intravenous clearance. Therefore, it is difficult to estimate clearance of therapeutic proteins when preparing first-in-man trials, because multiple clearance mechanisms are potentially involved in the clearance of these drugs.[32] In this study, only compounds that are known to be eliminated by ASGPRs (according to mechanistic studies reported in the literature) were investigated. Considering the relative success of the proposed single-species extrapolation model, it could be assumed that the model can only be applied in these circum-

stances. In other words, if it is not known which metabolic pathway is involved, then the model presented here cannot be applied to predict human clearance from a single preclinical species. Because there is still room for improvement, a full quantitative structure-activity relationship (QSAR)/*in silico* method remains to be established in a more quantitative way to identify the main metabolic pathway for therapeutic proteins, and the first attempts to develop QSARs for therapeutic proteins[33,34] may help to further drive future work. Coupled with QSAR/*in silico* methods predicting the metabolic pathway of a therapeutic protein, the proposed single-species method should be applicable in drug development while ASGPRs are identified. Similarly, if the main mechanism of clearance could be determined in one or more preclinical species in experimental studies (*in vitro* and/or *in vivo*), and if the mechanism is related to ASGPRs, this process should allow CL_H prediction before entering into human studies. Alternatively, the proposed physiological model could also be used to support mechanistic studies of clearance processes.[32] Consequently, the proposed model is an additional tool, which provides a meaningful testing tier to investigate whether a studied molecule is cleared by these receptors or not. In this case, inaccurate predictions of CL_H would have been expected in this study if the CL_H were strongly determined by receptors other than ASGPRs and/or some selected structural elements of these therapeutic proteins. Conversely, accurate predictions of CL_H *in vivo* for 14 extrapolation scenarios across species suggest some biological underpinning related to the abundance of ASGPRs in each species. Therefore, the results of this study enforce the role of ASGPRs in the mechanism of clearance of the five glycoproteins that were studied, which had originally been suggested by other authors.[3,19-27]

The principle of the proposed model is also applicable to other receptors such as LRPs and MRs, and to species such as monkeys, while the essential physiological input data on the abundance of these receptors and/or the QSAR method become available in the literature. The high usage of monkeys in drug development, irrespective of the high cost, is perhaps not surprising. In this study, such a physiological model for monkeys has not been challenged, principally because the essential input data on the abundance of ASGPRs cannot be found in the literature.

A proof of concept for the proposed physiological model is that the interspecies differences in CL_H *in vivo* follow the interspecies differences in abundance of ASGPRs (i.e. the ISF values). For example, CL_H *in vivo* in humans and dogs is nearly the same, per kilogram of body weight (table I). Consequently, the abundance of ASGPRs per kilogram of body weight (the
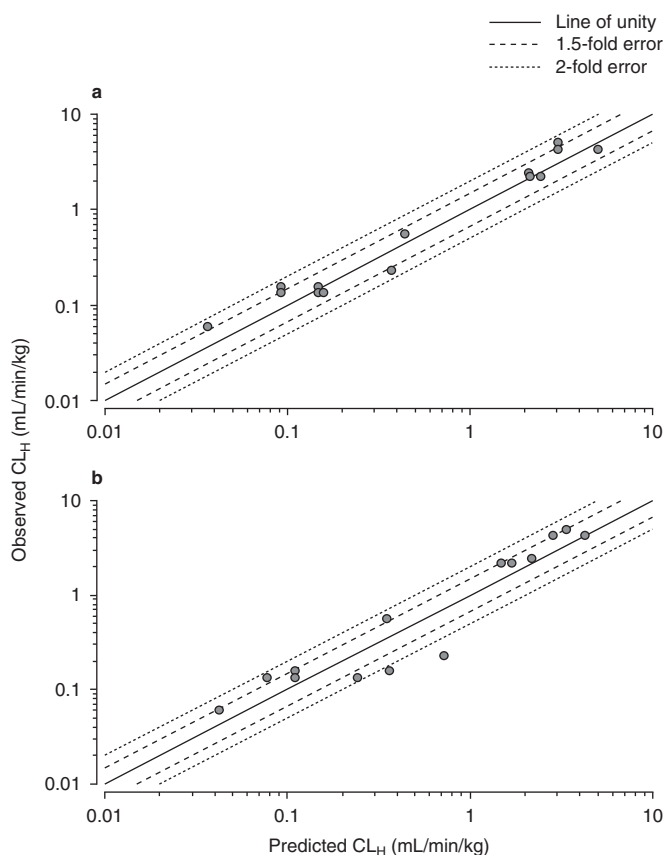

Fig. 2. Plot of predicted vs observed CL_H in the single-species models: (**a**) physiological model of this study (n = 14; r = 0.94); (**b**) empirical model [single-exponent theory] (n = 14; r = 0.97). The CL_H values are provided in table IV. **CL_H** = hepatic clearance.

Table V. Comparative assessment of the single-species model based on physiology and multiple-species allometry for prediction of CL_H in humans

Therapeutic protein	Species	Physiological model[a]		Multiple-species allometry[c]				
		predicted CL_H (mL/min/kg)	fold error[b]	exponent	r^2	predicted CL_H (mL/min/kg)	observed CL_H[d] (mL/min/kg)	fold error[b]
rhEPO	Mouse, rat, dog, monkey	0.092–0.157	0.94	0.69	0.96	0.073	0.135	0.54
Reteplase (BM 06022)	Rat, dog, monkey	3.05–5.02	0.96	0.89	0.99	4.45	4.3	1.03
Pamiteplase	Rat, dog, monkey	2.12–2.45	1.06	0.75	0.94	1.04	2.2	0.47

a A range of values is presented, since CL_H values in humans were predicted from more than one species (table IV) and calculated using equations 1 and 2. The input parameters on CL_H and global ISF values were obtained from tables I and III.

b Fold error of the difference between the predicted and observed values. Average values were used for the physiological model.

c Calculated using equations 4 and 5. The input parameters on CL_H and BW were obtained from table I.

d Obtained from table I.

BW = body weight; **CL_H** = hepatic clearance; **ISF** = interspecies scaling factor; **r^2** = squared correlation coefficient; **rhEPO** = recombinant human erythropoietin.

ISF) is also similar in these species, as previously mentioned (table II). An important finding is that standard empirical allometry did not perform better than the physiological model developed in this study. This has been verified using several statistical parameters and extrapolation scenarios. However, since the difference in predictivity between the prediction methods is sometimes small, it could be that both the proposed physiological model and allometry depend on body weight. In other words, it could be that the resulting exponent is comparable within these approaches if both the scaling factors (ISFs) and CL values are somehow related to the body weight. In this case, it seems that the values of the scaling factor used for ASGPRs (i.e. the ISFs) also correlate with the body weight, which could be a rational explanation. The resulting exponent and coefficient of correlation were 0.74 and 0.99, respectively, for the current dataset (data not shown). These compare well to multiple-species allometry following correlation of CL_H with the body weight (exponent ranges from 0.69 to 0.89, and coefficient of correlation ranges from 0.96 to 0.99) [table V]. The single-exponent theory presented a single exponent of about 0.8.[6] In essence, the proposed single-species model highlights the role of additional physiological determinants of the liver (e.g. the abundance of ASGPRs) that appear to be related to the body weight, which appears to influence the clearance of the selected therapeutic proteins. Scientifically, this is of great importance, since a more in-depth analysis of the role of physiology on CL_H has been conducted in this study.

The values of the resulting exponent are comparable but not all identical. Consequently, a single (unique) value could not be identified across the studied methods and compounds. This is not in total accordance with the single-exponent theory.[6] In this context, Mahmood[8] also observed relatively different exponent values across compounds in multiple-species allometry. However, it could be that the variability in the observed exponents depends on which datasets of clearance values are used,[3] or it could be that for specific compounds, the clearance mechanisms are not conserved across species.

The concept of single-species scaling was first introduced for small molecules, and later for some therapeutic proteins, as mentioned.[6-8,35] In this study, we have concisely challenged this concept with compound examples. Irrespective of the number of compounds that were studied, use of multiple species did not demonstrate a significant gain in accuracy compared with the single-species concept for the selected compounds that are cleared by ASGPRs (tables IV and V). This is in accordance with the literature.[6-8,35,36] It is considered important from the perspective of prediction of clearance in drug discovery, since clearance data in only one species could potentially be used to predict clearance in another species (e.g. humans).

Although the findings of the present study were established in a dataset using five glycoproteins, based on the availability of preclinical and clinical data, it still needs to be considered limited. However, it is worth mentioning that several (14) extrapolation scenarios across species were challenged. Nevertheless, it is difficult to be fully confident in the ability to compare data analysis across compounds, and this may influence confidence in the reliability of the conclusions presented here. Consideration of more compounds may change the conclusion of this study. However, while it is acknowledged that this could be important, it appears that the current physiological model performed relatively well compared with allometry. On a separate note, throughout this study, analysis of the current dataset of drugs was limited to prediction of the mean, without consideration of sensitivity, variability and

uncertainty. Although these issues are beyond the scope of the present study, analysis of sensitivity, variability and uncertainty would apply not only to the current physiological model but also to the conventional approaches that are used for allometry. In the present study, all approaches were compared by using the same datasets, at least limiting the variability of the datasets. Once the issues of sensitivity, variability and uncertainty have been addressed, it will be feasible to assess predictions, and the risks associated with uncertain predictions, more objectively.

It is well accepted that interspecies scaling works best for compounds that follow linear pharmacokinetics.[1-8] Therefore, use of the current physiological model of clearance should be less successful for compounds that do not follow this rule. In other words, as the clearance process predicted for ASGPRs is assumed to be linear with time and dose, compounds that follow nonlinear pharmacokinetics could not be considered by the present approach. In this context, rNIF has linear pharmacokinetics in rats but not in dogs.[27] Two mediated elimination pathways occur in dogs – namely, ASGPR and CD11b (single-pass type-1 α). For this reason, the clearance determined at the lowest dose was investigated particularly for the rat-to-dog extrapolation scenario, which was successfully predicted. Similarly, alteration of bone marrow function results in a significant change in rhEPO pharmacokinetics, indicating that bone marrow can also be a route of rhEPO elimination for low doses and may contribute to its non-linear pharmacokinetics via receptor-mediated endocytosis.[3] Despite the non-linear pharmacokinetic behaviour of rhEPO, CL_H was quite well correlated with the body weight,[3] as well as being accurately predicted (in this study) [tables IV and V] at the doses for which clearance data were reported in healthy subjects.[19]

In predicting CL_H, it is possible that interspecies differences in binding to plasma proteins have no impact on the therapeutic proteins that were studied. This is particularly true if the magnitude of binding of these macromolecules is relatively similar in each species. In the absence of definitive data in that domain, this assumption cannot be verified. Therefore, further clinical studies are necessary to also examine the impact of plasma protein binding on the scaling of clearance of the current compounds.[24]

Conclusions

A singles-species model, using liver physiology, has been developed for prediction of CL_H in animals and humans. According to the current findings, interspecies scaling of clearance of glycoprotein derivate therapeutics that are eliminated by ASGPRs seems to be governed by interspecies differences in the abundance of these receptors. Therefore, the physiological model is appropriate only for glycoprotein derivate therapeutics that are principally eliminated by ASGPRs. Superior or quite similar levels of accuracy were obtained with this model compared with standard allometry. Overall, the proposed physiological model is an additional tool, which should facilitate investigation and prediction of human clearance of specific glycoproteins solely on the basis of clearance data determined in a single preclinical species.

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