Estimating the predictive validity of diabetic animal models in rosiglitazone studies

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ABSTRACT

For therapeutic studies, predictive validity of animal models - arguably the most important feature of animal models in terms of human relevance - can be calculated retrospectively by obtaining data on treatment efficacy from human and animal trials. Using rosiglitazone as a case study, we aim to determine the predictive validity of animal models of diabetes, by analyzing which models perform most similarly to humans during rosiglitazone treatment in terms of changes in standard diabetes diagnosis parameters (glycosylated hemoglobin (HbA1c) and fasting glucose levels). A further objective of this article is to explore the impact of four covariates on the predictive capacity: i) diabetes induction method, ii) drug administration route, iii) sex of animals, and iv) diet during the experiments.

Despite the variable consistency of animal species-based models with the human reference for glucose and HbA1c treatment effects, our results show that glucose and HbA1c treatment effects in rats agreed better with the expected values based on human data than in other species. Induction method was also found to be a substantial factor affecting animal model performance.

The study concluded that regular reassessment of animal models can help to identify human relevance of each model and adapt research design for actual research goals

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INTRODUCTION

Although animal research is considered to be a central element of contemporary biomedical science and arguably has contributed greatly to the understanding of disease mechanisms and development of treatments, the predictive validity of different animal models is generally assumed and rarely measured. Having information on this feature of animals is crucial especially for therapeutic studies. The predictive validity of animal models which means 'to what extent research data from animals can predict human response to particular drugs' can be calculated retrospectively, after obtaining data on treatment efficacy from humans and animals.

Even though only human-animal comparative studies can produce evidence on human relevance of animal models and justify their use from a scientific point of view, relatively few studies have addressed their methodology. Several animal studies speculate on the translatability of results from animal studies by assuming on the validity of animal models. However, unique characteristics of animals models deserve special consideration on whether results can be translated to humans or not [1]. As Garth Whiteside has noted, comparison of large datasets from preclinical efficacy studies and human trials is a complex matter, and the predictive capacity of these models cannot be described as "worked", "didn't work", or "failed"; deeper analysis is needed [2].

To evaluate the predictive validity of animal models, one may opt for 1) assessing the predictive validity of a single or very few models by comparing treatment effects of series of interventions (e.g. administration of anti-diabetic drugs) in that/those animal model(s) and humans, or 2) assessing the predictive validity of several animal models by comparing the treatment effect of a particular intervention in those animals and humans. In either case, this comparison should be based on quantitative data that allow correlations between humans and different animal species to be calculated. This requires access to data where the same quantitative information (outcomes) is available for treatment effects both in animal and human subjects. Although research outcomes of an identical nature are regularly reported in human and animal studies, any study selection effort entails the setting of minimum criteria for study design quality. Furthermore, data availability is also an issue.

The quality of the data has a huge impact on the conclusions what can be drawn but raw data are rarely presented. The ideal human data for the calculation would be complete data from clinical trials. Due to the recent tight regulations on clinical trials (e.g. requirement of authorization [3] and prior registration [4]), transparency on the conduct and results of clinical trials has been improved. However, there are still problems with study design and publication from these trials. Regarding design, a widely discussed issue among others is the obscure management of missing data [5]. Additionally, we know that not all studies get published in their entirety after the clinical trials [6]; what is more, what does get published may be different from in-house interpretation and more likely to make a drug look favorable [7].

Also for animal data, it is increasingly evident that shortcomings in research design and publication bias resulting from selective publishing of desirable results are the cause of overestimated treatment effects [8, 9]. The retrospective evaluation of the predictive validity of animal models is further complicated by statistical weaknesses. Animal studies regularly report data from small samples of animals, and usually such studies are not repeated by independent third party laboratories.

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An additional challenge is that of comparing different species [10]. The efficient drug dose widely varies across species, mostly due to the pharmacokinetics of a particular drug being different from species to species [11]. It is commonly observed that small animals need to be administered larger doses (per kilogram body weight) as compared to big animals or humans to achieve similar pharmacological effects. For instance, about five-fold higher doses of prednisolone and caffeine have been reported for rats as compared to humans [12]. Providing cross-species comparisons of activity and toxicity of various drugs, two important methods are used for dosage conversions. One is based on per body surface area (BSA) calculation (mg/m^2) which is the method required by the FDA; the alternative method considers the daily expenditure of energy expressed per metabolically active mass (MAM) [12].

In the present paper, we propose a method for assessing the predictive validity of several animal models. Using rosiglitazone, a widely used pharmaceutical to treat type 2 diabetes mellitus, we aimed to provide data on the predictive validity of different animal models of diabetes. Type 2 diabetes was chosen since animal models are widely used in research into this disease [13] but their predictive validity has never been statistically studied. Rosiglitazone is an ideal case study to test the predictive validity of diabetes animal models: it is widely used in human patients and a preliminary PubMed search showed it to be the most commonly used pharmaceutical in animal studies into type 2 diabetes.

The main objective of this article is to determine the predictive validity of animal models of diabetes, by analyzing which models perform most similarly to humans during rosiglitazone treatment in terms of changes in standard diabetes diagnosis parameters (glycosylated hemoglobin (HbA1c) and fasting glucose (FG) levels). A further objective of this article is to explore the impact of four covariates on the predictive capacity of animal models. These covariates are methodological issues which often differ across studies, namely i) diabetes induction method, ii) drug administration route, iii) sex of animals, and iv) diet during the experiments.

MATERIAL AND METHODS

Literature review

Both human and animal studies were searched between September and December 2012. Studies reporting rosiglitazone monotherapies with information on glucose and/or HbA1c, published in English, were included. All references were downloaded and managed in Endnote. Two authors (OEV, NZs) assessed studies and extracted data into an excel table (Microsoft Office Excel 2007). Data on study design elements including time, route and dose of the drug administration, the species and strain of the animal, age and sex/gender of subjects, diets, diabetes induction method, outcomes (i.e. FG and HbA1c levels - number of observations, mean, variability measure) in each study group were extracted. In those papers where data was only reported graphically, a digital online ruler [14] was used to gain numerical values.

Animal studies were identified from Pubmed and Web of Science using the following algorithms. Pubmed search: ("animal experimentation"[MeSH Terms] OR "models, animal"[MeSH Terms]) AND "rosiglitazone"[Supplementary Concept] AND "blood glucose"[MeSH Terms]. Web of Science: Topic=(rosiglitazone) AND Topic=(blood glucose) AND Topic=(animal) AND Topic=(search filter suggested by Carlijn R Hooijmans[15]). The selection method with exclusion criteria is presented in Figure 1A. A total of 71 studies were included.
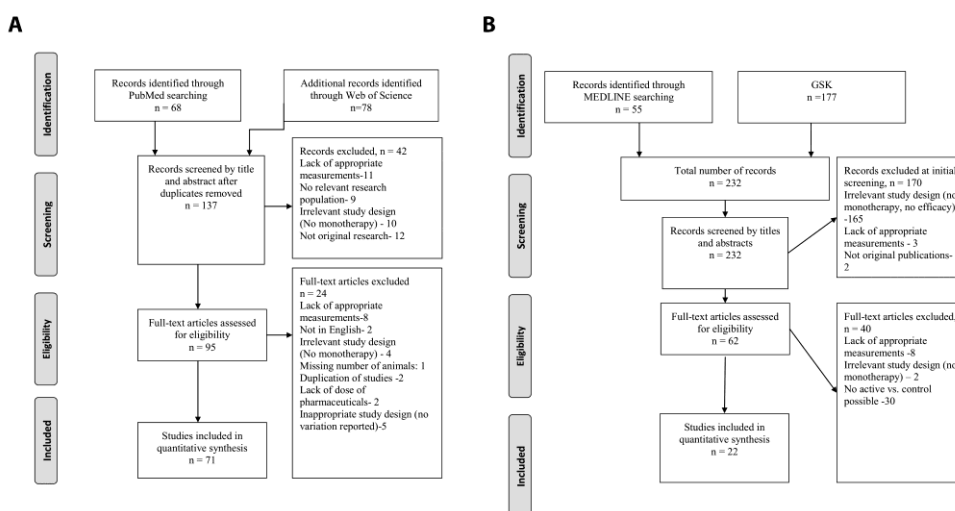

Figure. 1. Procedure on selection of studies. **(A)**: Procedure chart on how animal studies were selected. **(B):** Procedure chart on how human studies were selected.

Human studies were identified from three sources. A PubMed search that used the algorithm: "Blood Glucose"[Mesh] AND "Hemoglobin A, Glycosylated"[Mesh] AND "Diabetes Mellitus, Type 2/drug therapy"[Mesh] AND "rosiglitazone"[Supplementary Concept] AND "Thiazolidinediones/therapeutic use"[Mesh] AND "Randomized Controlled Trial"[ptyp] AND English[lang]. In addition, one study [16] from a published metaanalysis on the efficacy of thiazolidinediones (the class of drugs that include rosiglitiazone) in the Asian population [17] was included. Finally, all monotherapy studies were identified and included in the analysis from the website of the pharmaceutical company GlaxoSmithKline (GSK) who made all studies on rosiglitazone available through the company's website [18]. Details along with exclusion criteria are shown in Figure 1B.

Parameters and factors analyzed

To evaluate the predictive capacity of different animal models, human and animal glycosylated hemoglobin (HbA1c) and fasting glucose (FG) levels were selected as outcome measures. HbA1c is the primary laboratory test for diabetes in human studies and reflects average blood glucose for the preceding 60 to 90 days, whereas FG level is a very common parameter to monitor diabetes.

To evaluate the effect of certain factors which often differ across studies and may cause methodological issues, the following were considered: i) diabetes induction method; ii) drug

administration route; iii) sex of animals; and iv) diet during experiments. Rationale of selecting these covariates is given below.

i) A number of diabetic animal models have been developed over the years, based mostly on rodents; these models can be classified into two broad categories: 1) genetically induced spontaneous diabetes models and 2) experimentally induced (non-spontaneous) diabetes models [19]. The second consists of several subtypes: streptozotocin (STZ)/alloxan models, partial pancreatectomy models, high-fat (HF)/high-sucrose diet-fed models, HF diet-fed STZ models, and intrauterine growth retardation (IUGR) models [19].

ii) Several drug administration methods have been used to introduce the chemical substance. Oral administration, subcutaneous administration, and intra-peritoneal injection of substances are common procedures in scientific experiments [20].

iii) Since diet has a significant impact on diabetes induction and progress, diet is a crucial part of the experimentation, whether spontaneous or experimentally induced models are used [21].

iv) The sex of animals has a well-documented impact on diabetes mellitus progress, which may imply gender-specific clinical treatment of diabetes [22].

Statistical modeling and analysis

Information from all included studies was extracted and entered into a single database with as many observations for each study as the number of fasting glucose and/or HbA1c outcome measurements reported at distinct follow-up times in that study. Outcome level means and their standard errors were logarithmically transformed (natural base) using appropriate formulas to derive the expected value and standard error of the transformed variable working from the mean and standard error of the source variable. Treatment effect estimates at each observation time were calculated as the between-groups (rosiglitazone versus placebo) difference in transformed outcome levels. Treatment effect standard errors were calculated as the square root of the sum of group-specific squared standard errors. For the analysis of species effect, only rats and mice were included, whereas the remaining analyses were done on the complete dataset, including the two single studies on hamster and gerbil. For this reason animal strains in Figure 2A and 2B, are referred to as "Rat" and "Mouse".

Linear regression models were fitted on human observations for both outcomes. The dependent variable was the treatment effect; explanatory variables included dose, time into exposure (natural logarithm of week) complete with a quadratic derivative, and interaction terms between dose and time. Observations were weighted proportionally to their precision (reciprocal of squared standard error of treatment effect). The distribution of dose values was bimodal; a low vs high dose categorization cutoff of 6.5 mg was observed to coincide with a fairly wide gap between the two modes, close to the sample 50th percentile. To verify that this categorization did not cause substantial loss to model fit and/or changes to results and conclusions, the analysis was also completed using dose as a continuous variable, transforming rat doses (mg intake) to a human-comparable scale dividing by 2.25, and non-rat murine doses by dividing by 11.25, in accordance with FDA guidelines regarding interspecies dose conversion [3]. Model fit was evaluated using normality tests of residuals and Ramsey's regression specification-error tests, neither of which indicated any insufficiency of fit (all $P \geq 0.195$).

Coefficients derived from the human models were used to calculate expected values of non-human treatment effects. For each included study, and for each level of strain, diabetes induction method, special diet (yes or no), sex, and drug administration route within the study if applicable, differences between observed and expected treatment effects were squared, summed, divided by the number of measurement occasions, and taken the square root of. Values so derived were referred to as deviation scores.

The effects of factors raising methodological issues, such as species, induction method, diet, sex, and administration route were evaluated by comparing groups in terms of deviation scores. Basic unadjusted comparisons were made using simple linear regression. Adjusted effect estimates were obtained using multiple linear regression. Deviation scores were log-transformed to improve normality. Robust standard errors based on the clustering of observations within studies were used to make the estimation consistent with the presence of non-independence between observations coming from the same study. Explanatory variables with negligible effect estimates on both outcomes and no appreciable role as adjustment or interaction factors were eliminated to ensure model parsimony.

All statistical calculations and analyses were done using the software package Stata version 11. The detailed protocol of data collection and analysis is available as Supporting Information 1.

RESULTS

General description of dataset

As a result of rosiglitazone treatment, hyperglycemia ameliorated in both diabetic animals and T2DM patients. The dose used to efficiently reduce blood glucose and HbA1c levels in animals varied considerably across studies (6-20 mg/kg). Generally speaking, higher doses are used in animals than humans. In T2DM patients, rosiglitazone reduced fasting blood glucose and HbA1c levels at a daily dose of 4-8 mg. Animal studies differed from studies in human patients in terms of the age of study subjects: the initial age of human patients corresponded to late adulthood whereas that of animals represented adolescence and early adulthood. In animal studies the impact of treatment was detected by a comparison of glucose and HbA1c levels between treatment and placebo groups, whereas 30 out of 62 human studies presented data as compared to baseline measurements (see Figure 1), that could not be used for our analysis. Table 1 shows main characteristics of study subjects and glucose and HbA1c parameters from human and animal studies.

Table 1. Characteristics of subjects in the studies.

	Human	Rat	Mouse	Gerbil	Hamster
List of strains (number of studies, number of animals)	22, 3076	Brown Norway (1, 18)	(apo)E2 knock-in mice (1, 20)	gerbil (1, 32)	Syrian Golden (1, 30)
		Dahl SS/JrHsd (1, 20)	A-ZIP (1, 12)		
		Goto Kakizaki (1, 13)	BALB/c (1, 40)		
		LETO (1, 10)	C57BL6J (3, 70)		
		OLETF (1, 10)	DBA/2J (1, 63)		
		Ob-ZSF1 (1, 30)	FVB/N (1, 12)		
		Sprague Dawley (12, 293)	KKAgamma (1, 12)		
		Wistar (9, 189)	Ldlr-/-Apob100/100Lepob/ob (1, 23)		
		ZDF (14, 251)	MKR (1, 12)		
		Zucker lean (2, 32)	Swiss albino (2, 30)		
			apoE deficient (2, 96)		
			db/db (14, 214)		
			ob/ob (4, 52)		
initial age, weeks - weighted arithmetic mean (SD)	58.50 (8.93) years	8.59 (4.51)	7.05 (2.53)	16.00 (0.00)	9.00 (0.00)
Experimental time, weeks	27.09 (15.10)	5.47 (5.44)	8.53 (7.05)	2.00 (0.00)	5.00 (0.00)
Average dose used, mg/kg/day	4.87 (2.00)	6.49 (6.95)	11.59 (15.48)	20.00 (0.00)	7.15 (0.00)
Glucose level after treatment, mmol/l	8.44 (2.21)	10.58 (2.57)	13.28 (8.72)	6.34 (3.70)	3.50 (2.32)
HbA1c after treatment, %	7.69 (1.13)	4.71 (0.73)	7.19 (2.93)	no data	no data

Information provided in the table is based on all study subjects either treated with rosiglitazone or receiving no treatment, except for the rows "Glucose level after treatment" and "HbA1c after treatment" which include only treated groups. Means and standard deviations were pooled across studies by weighted averaging based on sample sizes, means and standard deviations reported for each study.

Comparing the consistency of animal models with the human reference for glucose and HbA1c treatment effects

All analyses shown below refer to models using dose as a categorical factor as no appreciable differences from results obtained with the continuous formulation were observed.

Rodent models roughly agreed with human data, especially for rats, but showed considerably varied accuracy in reflecting the efficacy of rosiglitazone in humans. For clarity, data on human-scaled values are provided in Table 2. The table shows how glucose and HbA1c levels differed in the rosiglitazone versus the placebo arms in humans at various time points into follow up. For example, in low dose treatment at three weeks, the average glucose level in the rosiglitazone arms was 85.9% of that in the placebo arms, or, in other words, a reduction of about 14% can be estimated as the treatment effect. For simplicity, data at median and terminal follow up time points were used to illustrate the tendencies.

Table 2. Ratios of glucose and HbA1c levels between the Rosiglitazone and placebo arms in human studies at different times into follow up.

	Fasting glucose level ratios, rosiglitazone vs placebo		HbA1c level ratios, rosiglitazone vs placebo	
low dose group, median follow-up	3 weeks	0.859 [0.478 to 1.545]	8 weeks	0.940 [0.908 to 0.974]
low dose group, end of follow-up	52 weeks	0.817 [0.691 to 0.965]	52 weeks	0.894 [0.811 to 0.985]
high dose group, median follow-up	3 weeks	1.056 [0.976 to 1.143]	8 weeks	1.027 [0.998 to 1.057]
low dose group, end of follow-up	52 weeks	0.870 [0.824 to 0.918]	52 weeks	0.992 [0.953 to 1.034]

Square brackets include 95% confidence intervals

Analysis of the 69 publications reporting studies with rats and mice (the single hamster and gerbil study excluded from this analysis) showed that the consistency of animal species-based models with the human reference for glucose and HbA1c treatment effects is highly variable. Glucose and HbA1c treatment effects in rats agreed better with the expected values based on human data than in mice, indicating that rat-based models may have greater consistency than those based on other species. Figure 2A shows that rats had significantly lower scores of deviation from the human reference than mice for glucose treatment effects during rosiglitazone treatment (means: 0.275 vs 0.594, respectively; P = 0.0023, unadjusted analysis). In case of HbA1c treatment effects, rats had the lowest deviation scores again (0.385 on average, vs 0.639 in mice), and the unadjusted difference was borderline significant (Figure 2B, P = 0.0446).

Strains

The question regarding which strain is the most appropriate to model the clinical efficacy of rosiglitazone could not be conclusively answered. In the analysed studies ten rat strains, eleven mouse strains, and two other species were used. Although SD rats and C57BL/6 mice seemed to be the most consistent animal models for the clinical efficacy of rosiglitazone, no statistical difference in deviation scores was observed between groups. The strain presenting the least consistent results for the clinical efficacy of rosiglitazone (in terms of both glucose and HbA1c treatment effects, with mean deviation scores 0.801 and 0.698, respectively) was the commonly used db/db mouse. Data on rat and mouse models are presented in Figure 2C.

A

n = 244, p = 0.0023

B

n = 69, p = 0.0446

C

Mouse strains: n = 113, p = 0.0000; rat strains: n = 131, p = 0.2199

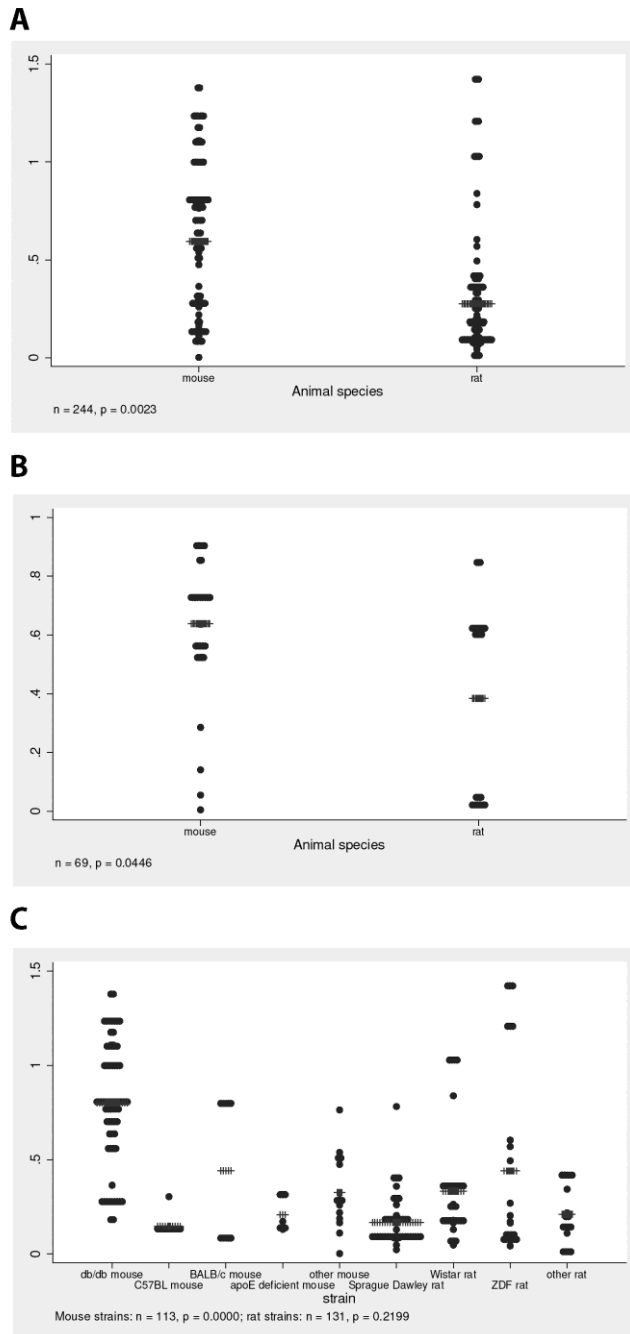**Figure. 2.** Comparing the animal models with the human reference for glucose and HbA1c treatment effects. **(A)**: Deviation scores for glucose treatment effects under rosiglitazone monotherapy in mice and rats. Horizontal lines of pluses indicate sample means; n denotes number of observations. **(B)**: Deviation scores for HbA1c treatment effects under rosiglitazone monotherapy in mice and rats. Horizontal lines of pluses indicate sample means; n denotes number of observations. **(C):** Deviation scores for glucose treatment effects under rosiglitazone monotherapy in rat and mouse strains. Horizontal lines of pluses indicate sample means. Strains rarely used in studies are pooled as "other mouse" and "other rat"; n denotes number of observations

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Impact of animal study covariates - induction method, drug administration route, sex of animals and applied diet

From the point of view of similarity to the human response to rosiglitazone, STZ induction was observed to be the most appropriate induction method, as presented in Figure 3A. STZ-induced diabetic animal models had the highest average consistency (mean deviation score: 0.237) with the human reference as evaluated in terms of the treatment effects of rosiglitazone on glucose levels. Performing a similar analysis for HbA1c was not practicable due to the low number of relevant studies.

Comparing the three administration methods, oral gavage was associated with the lowest deviance scores (mean: 0.369 for glucose, 0.449 for HbA1c). Of note, the number of observations for peritoneal administration was relatively small, and the unadjusted difference was only borderline significant for glucose (Figure 3B) and non-significant for HbA1c (Figure 3C).To test the hypothesis that humanized (high-sucrose, high-fat) or other diets could have an impact on diabetes onset and progress, and thereby on the consistency between animal and human models, deviation scores were compared across groups defined by high-sucrose, high-fat, high-sucrose-high-fat, and low-fat diets. No link was found between diet and the performance of animal models. Similarly, the sex of the animals was not observed to affect the deviation between animal and human models. Raw data associated with factors representing covariates are shown in Table 3.

Table 3. Description of factors that may cause methodological issues (covariates) in animal studies.

	Rat	Mouse	Gerbil	Hamster
Induction method (number of studies, number of animals)	SP (27, 350)	GM (6, 58)	SP (1, 16)	SP (1, 15)
	SP+STZ (1, 8)	GM+STZ (2, 24)		
	SP/diet (1, 10)	SP (21, 208)		
	alloxan (1, 5)	dexamethasone (1, 7)		
	dexamethasone (1, 6)	streptozotocin (5, 101)		
	diet (2, 14)			
	low protein IU diet (1, 7)			
	streptozotocin (13, 135)			
	surgery (1, 20)			
Administration route (number of studies, number of animals)	per os (14, 139) (1, 10)	per os (12, 129)	oral gavage (1, 16)	oral gavage (1, 15)
	oral gavage (24, 394)	oral gavage (15, 250)		
	intraperitoneal injection (1, 12)	intraperitoneal injection (2, 13)		
	unknown (1, 10)	unknown (1, 6)		
Diets (number of studies, number of animals)	high-NaCl (1, 10)	high-fat (6, 103)	high-energy (1, 16)	high-fat (1, 15)
	high-fat (7, 77)	low-fat (1, 5)		
	high-fat-high-sucrose (1, 8)	normal diet (21, 275)		
	normal diet (24, 265)	unknown diet (2, 15)		
	unknown diet (8, 195)			
Sex in animals in absolute numbers (male/female/both or unknown)	401/36/118	318/18/62	16/0/0	15/0/0

GM, genetically induced models; SP, spontaneous diabetes models; STZ, streptozotocin models; IU, intrauterine
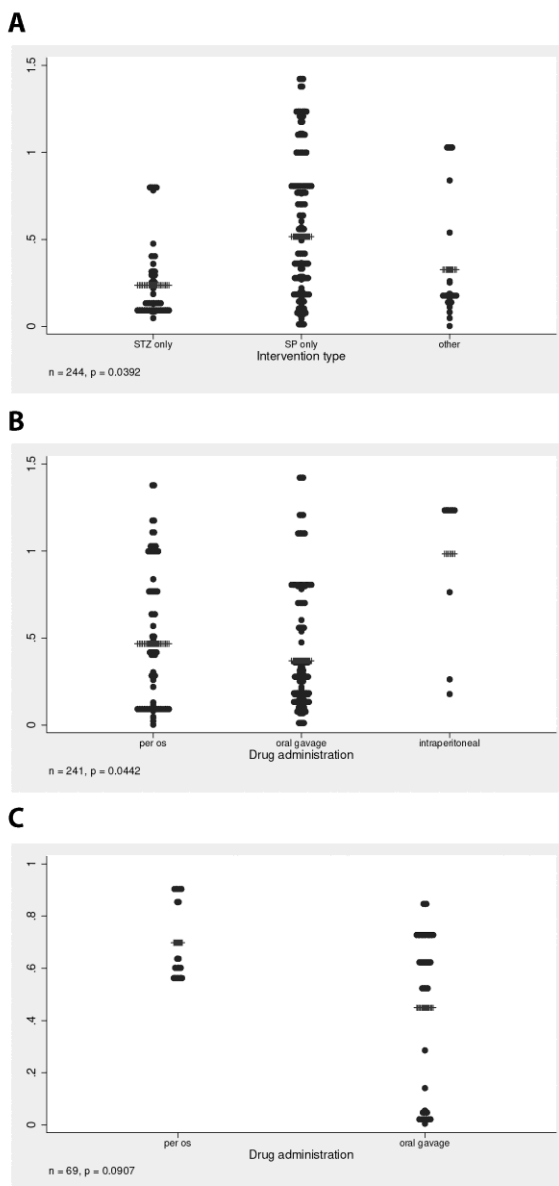
Figure. 3. Impact of animal study covariates. **(A)**: Deviation scores for glucose treatment effects under rosiglitazone monotherapy in groups by diabetes induction method. "STZ only" denotes the use of streptozotocin (STZ) only; "SP only", the absence of exogenous (non-spontaneous) induction methods; "other" denotes models that were not used frequently enough for individual analysis (alloxan models, partial pancreatectomy models, high-fat/high-sucrose diet-fed models, high-fat diet-fed STZ models, and intrauterine growth retardation models). Horizontal lines of pluses indicate sample means; n denotes number of observations. **(B)**: Deviation scores for glucose treatment effects under rosiglitazone monotherapy in groups by drug administration route. Horizontal lines of pluses indicate sample means; n denotes number of observations. **(C)**: Deviation scores for HbA1c treatment effects under rosiglitazone monotherapy in groups by drug administration route. Horizontal lines of pluses indicate sample means; n denotes number of observations.

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In multiple regression analysis, species, induction method, and drug administration route were found to be substantial factors of animal model performance (Table 4). Adjusted for induction method and administration route, rat models performed better: the difference in deviation scores between rats and other species was strongly significant for the fasting glucose outcome, although not quite for HbA1c. STZ as an induction method was found to better approximate the relationship between rosiglitazone exposure and treatment effect, observed in humans, than other methods, especially when treatment effect was assessed through HbA1c levels. The data also suggests that intraperitoneal administration may result in poorer consistency between human and animal models than per os administration.

Table 4. Additive effects of animal study factors on deviation scores of animal models

Factor	Contrast	Effect	95%CI		p
Outcome: fasting glucose					
Species	rat vs mouse	-0.790	-1.255	-0.325	0.001
Intervention	SP only vs STZ only	0.505	-0.086	1.097	0.093
Intervention	other vs STZ only	0.151	-0.722	1.023	0.731
Administration route	oral gavage vs per os	-0.188	-0.666	0.290	0.434
Administration route	intraperitoneal vs per os	0.650	0.130	1.169	0.015
Outcome: HbA1c					
Species	rat vs mouse	-1.577	-3.257	0.103	0.063
Intervention	SP only vs STZ only	3.179	1.020	5.339	0.008
Intervention	other vs STZ only	4.937	2.494	7.379	0.001
Administration route	oral gavage vs per os	-0.867	-2.131	0.397	0.159

Additive effects of animal study factors on deviation scores of animal models with respect to the human reference for the relationship between rosiglitazone exposure and treatment effect on follow-up levels of fasting glucose and HbA1c. CI, confidence interval

DISCUSSION

Clear understanding on the predictive validity of animal models is in those fields of applied drug testing where several animal models are used. In this study we presented a method to statistically evaluate the predictive validity of animal model studies. Using rosiglitazone in diabetes as a case study and comparing treatment effects between human and animal studies, we showed that studies in rats are better predictors of results in humans than other animal model studies. Agreement between human and animal studies was further affected by disease induction method and drug administration route. This was the first time that the concept of predictive capacity of animal models was systematically approached in type 2 diabetes pharmaceutical studies.

Our case study of rosiglitazone had two research questions: to compare treatment effect of rosiglitazone in human and animal models in order to identify which is more relevant to humans, and to understand the impact of experimental diet, induction method, sex, and administration route of rosiglitazone on the treatment effect of rosiglitazone.

According to the research question on the human relevance of animal models, our findings showed that although the consistency of animal species-based models with the human reference for glucose and HbA1c treatment effects are highly variable, glucose and HbA1c treatment effects in rats agreed better with the expected values based on human data than in other species; rats had significantly lower scores of deviation from the human reference than mice for glucose and HbA1c treatment effects. The question regarding which strain is the most appropriate to model the clinical efficacy of rosiglitazone could only be tentatively answered. There was no statistical difference in deviation scores observed between rat groups; among mouse strains, C57BL/6 showed the most consistent, while db/db showed the least consistent results.

Since models differ in physiological and genetic relevance, there is no single diabetic animal model which would fit for all scientific purposes; ideally, more than one species or strain are used in each study [13]. Three different approaches are used to evaluate the reliability of animal models: the first is phenomenological/pathophysiological similarity of the model to the syndrome it is imitating (face validity), the second compares the etiology of diseases in animal models and humans (construct validity), and the third approach refers to the ability of the model to respond to appropriate medications (predictive validity) [23]. The vast majority of reviews on T2DM animal models gives information on the models' face and construct validity, categorized by species. The characteristics of often used species such as murine models [24], or monkeys [25] or canines [26], or pigs [27] are widely discussed. As it was noted, very few studies on T2DM addressed the translatability of animal research results to humans and how to select animal models with "higher human relevance". A recent study has pointed out that genetic similarities between humans and certain species can be useful for appropriate model selection [28] and another study categorized mouse models by outcome measures that are used in the clinical practice of diabetic nephropathy [29].

A generally good correlation between human and animal experimental outcomes is often assumed in pharmacological studies, not considering the impact of the species effect. For example, the use of the leptin-deficient mouse (ob/ob) in type 2 diabetes research is widely recommended in any pharmaceutical research [30] but our case study does not prove "high predictive validity of this model" for rosiglitazone efficacy in humans. This example points out that if we want to understand how

reliable our animal models are in a particular situation, results must be (re)assessed in the light of human data.

Concerning our second research question, in multiple regression analysis, induction method, and drug administration route were studied, and the induction method was found to be a substantial factor affecting animal model performance. STZ as an induction method was associated with better approximation of the human relationship between rosiglitazone exposure and treatment effect than other methods, especially when treatment effect was ascertained through HbA1c levels. Although this result gives significant input for experimental design, it has to be underlined that these specific comparisons were complicated because HbA1c level, which is the primary outcome of human studies, was less frequently reported in animal studies [30].

There are several established methods for determining glycated hemoglobins[31] and many of them are used in rodents[32]. The relation between HbA1C and plasma glucose levels in diabetes animal models has also been well described[33]. However, in an ideal case, free plasma concentration of rosiglitazone could be compared between human and animal subjects, and identify how much of the drug is in the blood.

One of the strengths of this study is that to ensure sufficient coverage of relevant literature, it goes beyond traditional information sources; data from unpublished human studies have also been involved in the analysis. A very complex and long lawsuit filed against GSK started in 2007. One of the consequences of the legal action against GSK was that all studies performed by the company were made available through the company's website [18] and thus became available for our analysis. On the other hand, there are specific limitations to this work, such as the presence of different characteristics in human versus animal studies, which impeded the immediate comparability of the two datasets. One of these constrains was that the dose used to efficiently reduce blood glucose and HbA1c levels in animals varied considerably between studies (6-20 mg/kg). Generally speaking, higher doses are used in animals than humans. Additionally, in animal studies the impact of treatment was detected by a comparison of glucose and HbA1c levels between treatment and placebo groups; consequently, single-arm human studies that presented data as compared to baseline measurements, i.e. without a placebo control, could not be used in the analysis. Another problem was that the age of the study populations differed: the initial ages of human patients correspond to late adulthood whereas that of animals represents adolescence and early adulthood.

Animal models are unique in their predictive value for human drug efficacy. This study aimed to present how the predictive validity of animal models can be assessed retrospectively. Our method shows that regular reassessment of animal models helps to identify "human relevance of each model" and adapt research design for actual research goals. Although our findings are important, one should be careful with interpretation of results presented here; extrapolation of our results outside the thiazolidinedione class of drugs should be avoided.

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Supplementary material

PROTOCOL

Reviewers

Primary reviewer: Orsolya E Varga

Secondary reviewer: Noémi Zsíros

Involved in the analysis: László Kardos and I Anna S Olsson[1]

Review question/objective

The main objective of this article is to determine the predictive validity of animal models of diabetes, by analyzing which models perform most similarly to humans during rosiglitazone treatment in terms of changes in standard diabetes diagnosis parameters (glycosylated hemoglobin (HbA1c) and fasting glucose (FG) levels).

A further objective of this article is to explore the impact of four covariates on the predictive capacity of animal models. These covariates are methodological issues which often differ across studies, namely i) diabetes induction method, ii) drug administration route, iii) sex of animals, and iv) diet during the experiments.

Background

We will assess the predictive value – arguably the most important feature of animal models from the aspect of human relevance – of several animal models. Predictive validity can be calculated retrospectively by obtaining data on treatment efficacy from human and animal trials. In practice, the predictive value of different animal models is generally assumed and rarely measured. Using rosiglitazone as a case study, a widely used pharmaceutical to treat type 2 diabetes mellitus, we aim to provide data on the predictive validity of different animal models of diabetes. Similar analysis has not been published in this topic.

Although this analysis is important from both animal welfare and translational points of view, there are limitations. One of the main challenges is the poor quality of published research data from human and animal studies. Due to the recent tight regulations on clinical trials, transparency on the conduct and results of human clinical trials has been improved. However, there are still problems with study design and publication from these trials. Also for animal data, it is increasingly evident that shortcomings in research design and publication bias resulting from selective publishing of desirable results are the cause of overestimated treatment effects.

An additional challenge is that of comparing different species.

Selection criteria

Populations

Humans and animals

Intervention(s)/Indicator

Treatment with rosiglitazone (in monotherapy)

Comparators

Placebo

Outcomes

Glycosylated hemoglobin (HbA1c) and fasting glucose (FG) levels

Types of studies

The review will consider original research studies on the treatment effect of rosiglitazone

Planned search strategy

The search strategy aims to find both published and unpublished studies.

Two reviewers perform a first-stage screening of titles and abstracts based on the research question and its study design, sample, intervention, and outcome to be studied. Two reviewers will perform the second-stage screening of selected full-text articles.

Inclusion criteria: animal/human research (original studies), treatment effect of rosiglitazone, outcome measure is given in not-manipulated data of fasting glucose levels and/or HbA1c.

Exclusion criteria: no control group, not animal study, no data from outcome measures, rosiglitazone was not tested in monotherapy, design problems (eg no variation), not in English.

Studies published in English will be considered for inclusion in this review. Studies published to December 2012 will be considered for inclusion in this review.

The databases to be searched include:

MEDLINE, Web of Science.

The search for unpublished studies will include:

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GSK company site. Monotherapy studies will be identified and included in the analysis from the website of the pharmaceutical company GlaxoSmithKline (GSK) who made all studies on rosiglitazone available through the company's website.

Animal studies- Initial keywords to be used will be:

Animal studies will be identified from Pubmed and Web of Science using the following algorithms. Pubmed search: ("animal experimentation"[MeSH Terms] OR "models, animal"[MeSH Terms]) AND "rosiglitazone"[Supplementary Concept] AND "blood glucose"[MeSH Terms]. Web of Science: Topic=(rosiglitazone) AND Topic=(blood glucose) AND Topic=(animal) AND Topic=(search filter suggested by Carlijn R Hooijmans:

Topic=(rosiglitazone) AND Topic=(blood glucose) AND Topic=(animal) AND Topic=(animal OR animals OR pisces OR fish OR fishes OR catfish OR catfishes OR sheatfish OR silurus OR arius OR heteropneustes OR clarias OR gariepinus OR fathead minnow OR fathead minnows OR pimephales OR promelas OR cichlidae OR trout OR trouts OR char OR chars OR salvelinus OR salmo OR oncorhynchus OR guppy OR guppies OR millionfish OR poecilia OR goldfish OR goldfishes OR carassius OR auratus OR mullet OR mullets OR mugil OR curema OR shark OR sharks OR cod OR cods OR gadus OR morhua OR carp OR carps OR cyprinus OR carpio OR killifish OR eel OR eels OR anguilla OR zander OR sander OR lucioperca OR stizostedion OR turbot OR turbots OR psetta OR flatfish OR flatfishes OR plaice OR pleuronectes OR platessa OR tilapia OR tilapias OR oreochromis OR sarotherodon OR common sole OR dover sole OR solea OR zebrafish OR zebrafishes OR danio OR rerio OR seabass OR dicentrarchus OR labrax OR morone OR lamprey OR lampreys OR petromyzon OR pumpkinseed OR pumpkinseeds OR lepomis OR gibbosus OR herring OR clupea OR harengus OR amphibia OR amphibian OR amphibians OR anura OR salientia OR frog OR frogs OR rana OR toad OR toads OR bufo OR xenopus OR laevis OR bombina OR epidalea OR calamita OR salamander OR salamanders OR newt OR newts OR triturus OR reptilia OR reptile OR reptiles OR bearded dragon OR pogona OR vitticeps OR iguana OR iguanas OR lizard OR lizards OR anguis fragilis OR turtle OR turtles OR snakes OR snake OR aves OR bird OR birds OR quail OR quails OR coturnix OR bobwhite OR colinus OR virginianus OR poultry OR poultries OR fowl OR fowls OR chicken OR chickens OR gallus OR zebra finch OR taeniopygia OR guttata OR canary OR canaries OR serinus OR canaria OR parakeet OR parakeets OR grasskeet OR parrot OR parrots OR psittacine OR psittacines OR shelduck OR tadorna OR goose OR geese OR branta OR leucopsis OR woodlark OR lullula OR flycatcher OR ficedula OR hypoleuca OR dove OR doves OR geopelia OR cuneata OR duck OR ducks OR greylag OR graylag OR anser OR harrier OR circus pygargus OR red knot OR great knot OR calidris OR canutus OR godwit OR limosa OR lapponica OR meleagris OR gallopavo OR jackdaw OR corvus OR monedula OR ruff OR philomachus OR pugnax OR lapwing OR peewit OR plover OR vanellus OR swan OR cygnus OR columbianus OR bewickii OR gull OR chroicocephalus OR ridibundus OR albifrons OR great tit OR parus OR aythya OR fuligula OR streptopelia OR risoria OR spoonbill OR platalea OR leucorodia OR blackbird OR turdus OR merula OR blue tit OR cyanistes OR pigeon OR pigeons OR columba OR pintail OR anas OR starling OR sturnus OR owl OR athene noctua OR pochard OR ferina OR cockatiel OR nymphicus OR hollandicus OR skylark OR alauda OR tern OR sterna OR teal OR crecca OR oystercatcher OR haematopus OR ostralegus OR shrew OR shrews OR sorex OR araneus OR crocidura OR russula OR european mole OR talpa OR chiroptera OR bat OR bats OR eptesicus OR serotinus OR myotis OR dasycneme OR daubentonii OR pipistrelle OR pipistrellus OR cat OR cats OR felis OR catus OR feline OR dog OR dogs OR canis OR canine OR canines OR otter OR otters OR lutra OR badger OR

badgers OR meles OR fitchew OR fitch OR foumart or foulmart OR ferrets OR ferret OR polecat OR polecats OR mustela OR putorius OR weasel OR weasels OR fox OR foxes OR vulpes OR common seal OR phoca OR vitulina OR grey seal OR halichoerus OR horse OR horses OR equus OR equine OR equidae OR donkey OR donkeys OR mule OR mules OR pig OR pigs OR swine OR swines OR hog OR hogs OR boar OR boars OR porcine OR piglet OR piglets OR sus OR scrofa OR llama OR llamas OR lama OR glama OR deer OR deers OR cervus OR elaphus OR cow OR cows OR bos taurus OR bos indicus OR bovine OR bull OR bulls OR cattle OR bison OR bisons OR sheep OR sheeps OR ovis aries OR ovine OR lamb OR lambs OR mouflon OR mouflons OR goat OR goats OR capra OR caprine OR chamois OR rupicapra OR leporidae OR lagomorpha OR lagomorph OR rabbit OR rabbits OR oryctolagus OR cuniculus OR laprine OR hares OR lepus OR rodentia OR rodent OR rodents OR murinae OR mouse OR mice OR mus OR musculus OR murine OR woodmouse OR apodemus OR rat OR rats OR rattus OR norvegicus OR guinea pig OR guinea pigs OR cavia OR porcellus OR hamster OR hamsters OR mesocricetus OR cricetulus OR cricetus OR gerbil OR gerbils OR jird OR jirds OR meriones OR unguiculatus OR jerboa OR jerboas OR jaculus OR chinchilla OR chinchillas OR beaver OR beavers OR castor fiber OR castor canadensis OR sciuridae OR squirrel OR squirrels OR sciurus OR chipmunk OR chipmunks OR marmot OR marmots OR marmota OR suslik OR susliks OR spermophilus OR cynomys OR cottonrat OR cottonrats OR sigmodon OR vole OR voles OR microtus OR myodes OR glareolus OR primate OR primates OR prosimian OR prosimians OR lemur OR lemurs OR lemuridae OR loris OR bush baby OR bush babies OR bushbaby OR bushbabies OR galago OR galagos OR anthropoidea OR anthropoids OR simian OR simians OR monkey OR monkeys OR marmoset OR marmosets OR callithrix OR cebuella OR tamarin OR tamarins OR saguinus OR leontopithecus OR squirrel monkey OR squirrel monkeys OR saimiri OR night monkey OR night monkeys OR owl monkey OR owl monkeys OR douroucoulis OR aotus OR spider monkey OR spider monkeys OR ateles OR baboon OR baboons OR papio OR rhesus monkey OR macaque OR macaca OR mulatta OR cynomolgus OR fascicularis OR green monkey OR green monkeys OR chlorocebus OR vervet OR vervets OR pygerythrus OR hominoidea OR ape OR apes OR hylobatidae OR gibbon OR gibbons OR siamang OR siamangs OR nomascus OR symphalangus OR hominidae OR orangutan OR orangutans OR pongo OR chimpanzee OR chimpanzees OR pan troglodytes OR bonobo OR bonobos OR pan paniscus OR gorilla OR gorillas OR troglodytes)

Human studies-- Initial keywords to be used will be:

A PubMed search will be used the algorithm: "Blood Glucose"[Mesh] AND "Hemoglobin A, Glycosylated"[Mesh] AND "Diabetes Mellitus, Type 2/drug therapy"[Mesh] AND "rosiglitazone"[Supplementary Concept] AND "Thiazolidinediones/therapeutic use"[Mesh] AND "Randomized Controlled Trial"[ptyp] AND English[lang].

Planned data extraction

Two coauthors will download all references into Endnote. Data will be extracted into an excel table (Microsoft Office Excel 2007). Data will be extracted on study design elements: the time, route and dose of the drug administration, the species and strain of the animal, age and sex/gender of subjects, diets, diabetes induction method; and on outcomes, i.e. FG and HbA1c levels (number of observations, mean, variability measure) in each study group.

Where data would be reported graphically, digital online ruler will be used.

All data will be manually extracted by two reviewers.

Quality appraisal

To reduce bias appropriate research terms will used (eg. Randomized Controlled Trial), research papers with no control groups will be excluded and observations will be weighted proportionally to their precision (reciprocal of squared standard error of treatment effect) during analysis.

Data analysis and synthesis

Quantitative data will be extracted from papers included in further modeling (human-animal comparison). The analysis will be stratified according to i) species and strains ii) diabetes induction method, iii) drug administration route, iv) sex of animals, and v) diet during the experiments. Data will be analyzed with STATA and the significance level will be set at p<0.05.

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