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Overlapping roles of NADPH Oxidase 4 (Nox4) for diabetic and gadolinium-based contrast agent-induced systemic fibrosis-Animal Equivalent Dosing of Gadolinium-based contrast agents Supplementary Figure 1

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SUPPLEMENTARY MATERIAL

Animal equivalent dosing of gadolinium-based contrast agents.

A reasonable model for gadolinium retention and renal elimination of pharmaceuticals with a volume of distribution largely in the plasma compartment is referenced by Drs. Seishiro Hirano and Kazuo T. Suzuki in *Environmental Health Perspectives* (1):

$$Retention = Ae^{(-\frac{0.693}{T_a})t} + Be^{(-\frac{0.693}{T_b})t} + Ce^{(-\frac{0.693}{T_c})t}$$

Where t is time, T_a, T_b, and T_c are the half-lives of fast, intermediate, and slow phases of elimination, and A, B, and C are the proportions (adding to 100%) that represent each of those compartments. Non-protein-bound gadolinium-based contrast is cleared similar to non-protein-bound iodinated contrast, and this follows inulin clearance (which is why iothalamate can be used to approximate *true* glomerular filtration rate). This half-life for mice is less than 5 minutes. Gadolinium-based contrast agent exposure, although at a high concentration, is *rapidly* eliminated from mice (**Supplementary Figure 1**).

Interspecies allometric scaling—the empirical approach of exchanging drug dose between species is based on the normalization of dose to body surface area—is a widely studied area of clinical pharmacology. In 2005, the FDA published a Guidance for Industry (2) for comparative drug dosing. Dose by factor method is an empirical approach that utilizes the no observed adverse effect levels (NOAEL) of a drug from preclinical studies for the estimation of a human equivalent dose (HED). The dose by factor method applies an exponent for body surface area that accounts for the difference in metabolic rates allowing for dose conversion for non-human species. Similarly, the animal equivalent

dose (*AED*) can be calculated from the basis of body surface area by dividing or multiplying the human dose (mmol/kg) by the correction factor (K_m). The K_m ratio is estimated by dividing the average body weight (kg) of the species to its body surface area (m^2). *AED* (mg/kg) is calculated by the following:

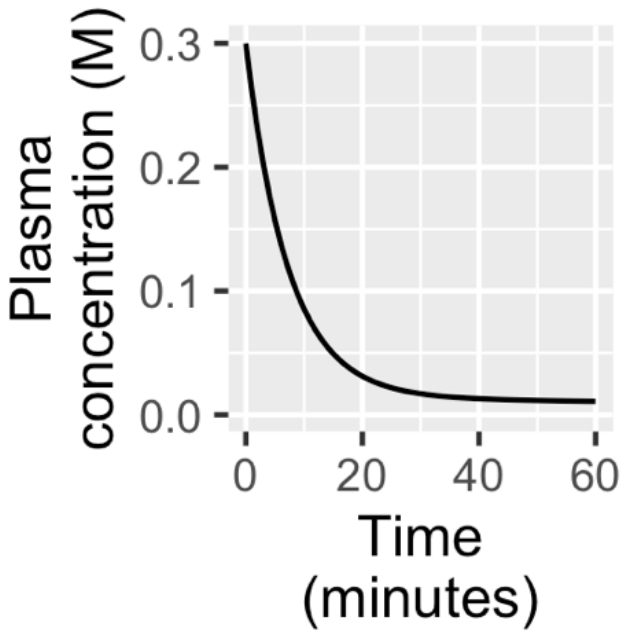
$$AED = Dose_{(Human)} \times K_m,$$

(with the human dose being in mmol/kg). To convert to mouse dose, the human dose (mmol/kg) is multiplied by 12.3 or divided by 0.081 (3).

The label dose of most of the gadolinium-based contrast agents is 0.05 to 0.1 mmol/kg. However, industry preclinical safety studies report a standard gadolinium-based contrast agent dose of 2.5 mmol/kg, 20 doses over a 4-week period (4). Pharmacokinetics of these agents, including the linear agent gadodiamide (Omniscan) and macrocyclic agent gadoterate meglumine (Dotarem), were evaluated using doses up to 0.3 mmol/kg (Omniscan [package insert]. Marlborough, MA: GE Healthcare Inc; 2010, Dotarem [package insert]. Princeton, NJ: Guerbet LLC; 2017]). Multiple studies outside of human patient safety trials have exceeded these ranges. Dosing and tolerability of gadobutrol (Gadavist) were assessed using single intravenous administrations of low concentrations, ranging from 0.04 – 0.4 mmol/kg, and high concentrations, ranging from 0.3 – 0.5 mmol/kg (5). The prognostic role of cardiac magnetic resonance imaging in patients with dilated cardiomyopathy has been evaluated with doses ranging from 0.15 mmol/kg (6) to 0.2 mmol/kg (7). Gadolinium-based contrast-enhanced magnetic resonance angiography, an off-label practice, has been used to assess renal vascular disease at doses up to 0.2 mmol/kg (8) and diagnose pulmonary embolism at

approximately 0.3 mmol/kg (8). Multiple sclerosis trials have used up to 0.3 mmol/kg of Magnevist (with the findings that gadolinium induces hypophosphatemia, long-term cranial bone abnormalities (9) and permanent retention in brain grey matter (10)). Other off-label uses of gadolinium-based contrast agents have been recorded, including 0.4 mmol/kg (up to 60 mL) for cerebral perfusion protocols (11).

The dose in our *in vivo* model of 2.5 mmol/kg per day aiming for 20 doses over one month was founded on that of investigators from Bayer Pharmaceuticals (12). This dose is in the range of that used in the rodent pharmacokinetic studies leading to the approval of most of these gadolinium-based contrast agents (13). Therefore, 2.5 mmol/kg has been used in many of the preclinical rodent trials prior to 2006, and precisely what was used in our studies with rats (14-17). The purpose of our studies is to replicate the findings of systemic fibrosis (16). Repeated dosing of gadolinium-based contrast agents is not uncommon. There are many recent published reports of multiple gadolinium-based contrast-enhanced MRI examinations (18, 19). Of note a recent study focused on brain tissue gadolinium retention in pediatric patients following repeated exposures of linear and macrocyclic gadolinium-based contrast agents (20). This finding is of great concern as the long-term effects of retained gadolinium is generally unknown but is of tantamount concern in children, given their state of development and expected longer period of exposure. A patient with *normal renal function* subject to 61 gadolinium doses (21) developed gadolinium retention in the skin with CD34 positivity (i.e., a histologic criterion for nephrogenic systemic fibrosis), and joint contractures (a major criterion for nephrogenic systemic fibrosis).



Supplementary Figure 1. **Modeling plasma gadolinium-based contrast agent concentrations based on the Hirano Suzuki equation**. The half-life of the rapid elimination phase (set at 95%) is assumed to be 5 minutes for the mouse—i.e., the same as inulin—with the intermediate and delayed phases (2.5% contributions each) of 50 and 500 min. In our model, 96.4% of the gadolinium-based contrast is eliminated within one hour of administration and 99.5% eliminated within 24 hours.

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