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Distribution profile of gadolinium in gadolinium chelate-treated renally-impaired rats: Role of pharmaceutical formulation

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ABSTRACT

While not acutely toxic, chronic hepatic effect of certain gadolinium chelates (GC), used as contrast agent for magnetic resonance imaging, might represent a risk in renally-impaired patients due to free gadolinium accumulation in the liver. To answer this question, this study investigated the consequences of the presence of small amounts of either a soluble gadolinium salt ("free" Gd) or low-stability chelating impurity in the pharmaceutical solution of gadoteric acid, a macrocyclic GC with high thermodynamic and kinetic stabilities, were investigated in renally-impaired rats. Renal failure was induced by adding 0.75% adenine in the diet for three weeks. The pharmaceutical and commercial solution of gadoteric acid was administered (5 daily intravenous injections of 2.5 mmol Gd/kg) either alone or after being spiked with either "free" gadolinium (i.e., 0.04% w/v) or low-stability impurity (i.e., 0.06 w/v). Another GC, gadodiamide (low thermodynamic and kinetic stabilities) was given as its commercial solution at a similar dose. Non-chelated gadolinium was tested at two doses (0.005 and 0.01 mmol Gd/kg) as acetate salt. Gadodiamide induced systemic toxicity (mortality, severe epidermal and dermal lesions) and substantial tissue Gd retention. The addition of very low amounts of "free", non-chelated gadolinium or low thermodynamic stability impurity to the pharmaceutical solution of the thermodynamically stable GC gadoteric acid resulted in substantial capture of metal by the liver, similar to what was observed in "free" gadolinium salt-treated rats. Relaxometry studies strongly suggested the presence of free and soluble gadolinium in the liver. Electron microscopy examinations revealed the presence of free and insoluble gadolinium deposits in hepatocytes and Kupffer cells of rats treated with gadoteric acid solution spiked with low-stability impurity, free gadolinium and gadodiamide, but not in rats treated with the pharmaceutical solution of gadoteric acid. The presence of impurities in the GC pharmaceutical solution may have long-term biological consequences.

1. Introduction

The identification of a causal link between gadolinium chelates (GCs), widely used as a contrast agent for magnetic resonance imaging (MRI), and nephrogenic systemic fibrosis (NSF), a highly debilitating disease occurring in patients with severe or end-stage renal failure (Abu-Alfa, 2011; Grobner, 2006; Marckmann et al., 2006), has prompted a substantial amount of studies aimed at clarifying the structure–toxicity relationship of these molecules (Idée et al., 2014; Morcos, 2011). The vast majority of published cases of NSF were associated with the non-ionic, linear GC gadodiamide (Heverhagen et al., 2014).

GCs are small molecular weight, extracellular space markers that enhance tissue contrast on magnetic resonance T_1-weighted

Abbreviations: ALAT, alanine aminotransferase; ANOVA, analysis of variance; ASAT, aspartate aminotransferase; BW, body weight; Ca, calcium; Ca-DTPA-BMA, calcium diethylene triamine pentaacetic acid bismethylamide; Ca-DTPA-BMEA, calcium diethylene triamine pentaacetic acid-N,N′-bis (methoxyethylamide); EDDA, ethylene diamine diorthohydroxyphenyl acetic acid; EELS, electron energy loss spectroscopy; EFTEM, energy-filtered transmission electron microscopy; EM, electron microscopy; Fe, iron; g, gram; GC, gadolinium chelate; Gd, gadolinium; HES, haematoxylin–eosin–saffron; ICH, international conference of harmonisation; ICP–MS, inductively coupled plasma mass spectrometry; ID, injected dose; kg, kilogram; K_{therm}, thermodynamic stability constant; L, ligand; l, liter; Ln, lanthanide; LOD, limit of detection; LOQ, limit of quantification; M, metal; Mg, magnesium; MHz, megahertz; min, minute; ml, milliliter; mM, millimolar; mm, millimeter; MRI, magnetic resonance imaging; *n*, number; nM, nanomolar; nmol, nanomol; NSF, nephrogenic systemic fibrosis; P, phosphorus; r_1, longitudinal relaxivity constant; s, second; SD, standard deviation; T_1, longitudinal relaxation time; µM, micromolar; µm, micrometer; w/v, weight/volume.

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images by increasing the longitudinal relaxation rate of extracellular fluid protons ($1/T_1$) (Hao et al., 2012). They are widely used to enhance the diagnostic efficacy of MRI for the detection and characterisation of lesions and for the evaluation of perfusion and flow-related abnormalities. Contrast agents are considered as drugs by Health Authorities worldwide.

Due to its high acute toxicity (Palasz and Czekaj, 2000), the lanthanide (Ln) metal gadolinium (Gd) must be chelated with an appropriate ligand for its administration to patients (Port et al., 2008). Structurally, the GC ligand can be either linear or macrocyclic and the resulting chelate can be either 'ionic' or 'non-ionic' (i.e., neutral), depending on the number of the carboxyl groups on the polyaza-polycarboxylic ligand (Port et al., 2008). These chelates follow the mass action law. The stability of a metal chelate refers to the equilibrium between the metal (M) and its ligand (L). The chelate (ML) therefore follows the equation:

$$[M] + [L] \rightleftarrows [ML] \quad (1)$$

The stability of GC is commonly described using two concepts: (a) thermodynamic stability, which describes the strength of the link between the Gd and its ligand (expressed in terms of thermodynamic stability constant $\mathrm{Log}\,K_{\mathrm{therm}}$) and (b) kinetic stability, which refers to the rate at which dissociation of the GC occurs (characterised by the dissociation half-life ($T_{1/2}$) at acidic pH) (Port et al., 2008).

Most non-clinical and clinical studies available to date favour a causal role of dissociated Gd in tissues in the pathogenesis of NSF (Abraham et al., 2008; Fretellier et al., 2011b, 2012, 2013; Haylor et al., 2012; Idée et al., 2014; Pietsch et al., 2009; Sieber et al., 2008a,b,c; Wadas et al., 2010), although this hypothesis remains disputed by some authors (Grant et al., 2009; Wermuth and Jimenez, 2014). Basically, GCs differ in their ability to release dissociated Gd, with macrocyclic GCs being kinetically more stable than linear agents and linear ionic compounds being thermodynamically more stable than linear non-ionic GCs (Port et al., 2008). To prevent the possibility of GC dissociation during shelf life, certain compounds are formulated with an excess of free ligand added in the pharmaceutical solution (Idée et al., 2009).

Free gadolinium stimulate fibroblast proliferation, both in insoluble (Bleavins et al., 2012; Li et al., 2010) and soluble form (Bhagavathula et al., 2010; Varani et al., 2009). This effect is actually shared by all lanthanides (Jenkins et al., 2011). In addition to its proliferative effects on fibroblasts, because its ionic radius is close to that of Ca^{2+}, free Gd is a potent inhibitor of all physiological Ca^{2+}-dependent processes (e.g., blood coagulation, contraction of smooth and skeletal muscle, transmission of nerve impulses, etc.). It also inhibits the activity of certain enzymes such as Ca^{2+} or Mg^{2+}-activated adenosine triphosphatase (Idée et al., 2009; Palasz and Czekaj, 2000). Gadolinium ions are a potent inhibitor of Kupffer cell phagocytosis (Palasz and Czekaj, 2000).

In fact, following intravenous administration of GC for an MRI procedure, the body's exposure to free Gd can be the consequence of either tissue dissociation and/or administration of free Gd present in the pharmaceutical solution of the contrast agent. Although the latter may be unlikely due to stringent regulations, its consequences have never been investigated *in vivo*, to our knowledge. Another possibility is the *in vivo* dissociation of a Gd-chelating impurity, which may be produced during the pharmaceutical process and be present in the pharmaceutical solution.

The aims of this study were to evaluate the risk associated with the presence of soluble and "free" Gd or a low thermodynamic stability chelating impurity in the pharmaceutical solution of a thermodynamically stable GC, gadoteric acid, and to compare the distribution of the Gd metal in the body, especially the liver, the main target organ of free gadolinium. The commercial solution of a lower thermodynamic stability GC, gadodiamide was investigated as a positive control (Fretellier et al., 2013; Pietsch et al., 2009).

2. Material and methods

2.1. Animal model

All experimental procedures were performed in accordance with French regulations and in compliance with the European Union Directive 2010/63/EU on animal welfare. The experimental protocol was accepted by the internal ethics committee.

A total of 56 six-week-old male Wistar rats (Centre d'Elevage René Janvier, CERJ, Le Genest Saint Isle, France) weighing 200–220 g at arrival was used in the study (8 animals per group). The animals were housed two per cage at an ambient temperature of 22 ± 2 °C and a hygrometry of 45 ± 10%, with 12-h light and 12-h dark cycles. The rats were given free access to water and food (A04, SAFE, Augy, France) for 10 days of acclimatisation before the experiments began.

At the start of the study (Day 0), the animals were given a diet containing 0.75% adenine (SAFE) for 3 weeks (Day 0–Day 21) to induce severe renal failure and were then fed a standard diet (A04) for another 2 weeks (Fretellier et al., 2013). The rats were housed individually once the study began.

2.2. Products

We investigated the effects of unchelated and "free" Gd (using Gd acetate), the pharmaceutical solution of gadodiamide (linear and non-ionic GC), the pharmaceutical solution of gadoteric acid (macrocyclic and ionic GC) alone or spiked with "free" and soluble Gd (Gd acetate) or with low-stability Gd chelating impurity (mixture of Gd-EDDA and Gd-$(EDDA)_2$), in renally-impaired rats. The test solutions and doses are shown in Table 1. All test solutions

Table 1
Test solutions and doses.

Test solution	Source	Gd dose (mmol Gd/kg)
Isotonic saline (at 0.9%)	Lavoisier, Paris, France	0
Gadodiamide (Omniscan®) at 500 mM	General Electrics Healthcare, Chalfont St Giles, UK	2.5 mmol Gd/kg
Gadoteric acid (Dotarem®) at 500 mM	Guerbet, Villepinte, France	2.5 mmol Gd/kg
Gadoteric acid (Dotarem®) (500 mM) spiked with low stability impurity (EDDA-Gd/(EDDA)2-Gd) (0.06% w/v)	Guerbet, Villepinte, France; impurity synthesized at Guerbet Research & Innovation Department	2.5 mmol Gd/kg gadoteric acid) 0.009 mmol Gd/kg (from impurity) Total dose: 2.509 mmol Gd/kg
Gadoteric acid (Dotarem®) (500 mM) spiked with Gd acetate (0.04% w/v)	Guerbet, Villepinte, France	2.5 mmol Gd/kg (gadoteric acid) 0.013 mmol Gd/kg (Gd acetate) Total dose: 2.513 mmol Gd/kg
Gd acetate at 1 mM	Sigma–Aldrich, Saint Quentin-Fallavier, France	0.005 mmol Gd/kg
Gd acetate at 2 mM	Sigma–Aldrich, Saint Quentin-Fallavier, France	0.01 mmol Gd/kg