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# Safety profiles of gadolinium chelates in juvenile rats differ according to the risk of dissociation

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ABSTRACT

This study was designed to compare the safety of two gadolinium chelates (GCs), used as contrast agents for magnetic resonance imaging, in juvenile rats. Juvenile rats received five intravenous administrations (between postnatal day [PND] 4 and 18) of gadoteric acid (macrocyclic ionic GC), gadodiamide (linear nonionic GC) or saline, and sacrificed at PND 25. Gadodiamide induced mortality, alopecia and hyperpigmentation of dorsal skin. Two gadodiamide-treated rats presented severe epidermal and dermal lesions. No abnormal signs were detected following administration of gadoteric acid. Higher tissue gadolinium concentrations were found in the gadodiamide group compared to the gadoteric acid group. Dissociation of gadodiamide was observed in skin and liver, with the presence of dissociated and soluble gadolinium. In conclusion, repeated administration of gadoteric acid was well tolerated by juvenile rats. In contrast, gadodiamide induced significant toxicity and more marked tissue gadolinium retention (at least partly in the dissociated and soluble form).

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## 1. Introduction

Gadolinium is a highly toxic lanthanide [1]. Its acute toxicity appears to be related to its ionic radius close to that of $Ca^{2+}$ [1]. The $Gd^{3+}$ ion must therefore be chelated by an appropriate ligand to allow intravenous administration to patients as contrast agents for magnetic resonance imaging (MRI). Gadolinium chelates (GCs) are classified according to their chemical structure (linear or cyclic) and the ionic or nonionic nature of the ligand used to chelate $Gd^{3+}$. So-called "macrocyclic" GCs consist of a ligand forming a "cage" imprisoning $Gd^{3+}$ (such as gadoteric acid). "Linear" chelates are molecules in which Gd is associated with a ligand with an "open" structure (such as gadodiamide). The thermodynamic and kinetic stabilities of the various GCs differ according to these structural characteristics [1,2]. Thermodynamic stability describes the strength of the bond between Gd and its ligand. Kinetic stability defines the rate at which equilibrium between the Gd chelate and its dissociated component is reached. The kinetic stability of macrocyclic GCs is much higher than that of linear GCs and the thermodynamic stability of ionic GCs is generally higher than that of nonionic GCs [1–3]. GCs are not metabolized and are almost exclusively excreted by the kidneys by glomerular filtration [1].

MRI has become a major tool for pediatric patients for three main reasons: superior tissue characterization compared to computed tomography (CT), smaller injected volumes of contrast agent are required compared to CT, and no exposure to ionizing radiation,

*Abbreviations:* GC, gadolinium chelate; PND, postnatal day; MRI, magnetic resonance imaging; CT, computed tomography; NSF, nephrogenic systemic fibrosis; EMA, European Medicines Agency; FDA, Food and Drug Administration; ELISA, enzyme-linked immunosorbent assay; HES, hematoxylin-eosin-saffron; LOD, limit of detection; LOQ, limit of quantification; HPLC, high pressure liquid chromatography; CNS, central nervous system.

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which is crucial in children [4]. Contrast agent administration is necessary to allow better visualization and characterization of micro-vessels or lymph, for example, or to distinguish certain tumors. Contrast-enhanced MRI examinations are performed in neonates and young children whenever the following clinical indications [5–7] are present: neonatal tumors, evaluation of lesions detected on prenatal ultrasound, hemodynamically significant vascular malformations, congenitally acquired infections and congenital heart disease. Repeated MRI monitoring may also be necessary in some cases, as the frequency of follow-up may vary depending on the grade of the lesions, biological activity and treatment [8]. For example, in children with brain tumors, serial MRI is commonly performed every 3–6 months after surgery, as long as clinically necessary.

Although generally considered to be safe in the majority of patients, GCs can be associated with the development of a severe delayed adverse reaction, nephrogenic systemic fibrosis (NSF). NSF has only been diagnosed in patients with severe or end-stage renal failure [9]. Almost all cases of NSF induced by a single GC (so-called "unconfounded" cases), appear to be related to a specific category of GCs, linear GCs, and more particularly gadodiamide and gadopentetic acid [10], while almost no cases of confirmed NSF have been linked to the administration of other categories of GCs. In particular, no unconfounded case of NSF has been reported for gadoteric acid, evaluated in the present study. The youngest NSF pediatric published case was reported in a six-year-old child [11].

Anatomical development of the kidneys (nephrogenesis) in humans is complete at the 35th week of gestation [12] and is followed by a process of morphological and functional maturation of the kidneys to achieve an adult level of renal function around the age of two years [12]. Immaturity of renal function in neonates and infants may therefore result in increased systemic exposure, tissue retention of $Gd^{3+}$ and significant adverse effects.

The safety of GCs in pediatric patients is a matter of concern for radiologists and health authorities. Relatively few clinical safety data are available for GCs in the pediatric population. Moreover, nonclinical juvenile toxicity studies are rarely carried out to predict safety in the pediatric population [13,14]. A better understanding of the safety profile of GCs in this specific population is essential. The European Medicines Agency (EMA) has contraindicated the use of GCs considered to be associated with a high risk of NSF (gadodiamide, gadopentetic acid and gadoversetamide) in neonates under the age of 4 weeks. However, for GCs considered to be associated with a low risk (gadoteric acid, gadobutrol and gadoteridol) or intermediate risk of NSF (gadobenic acid), only a precaution for use has been issued in newborns up to the age of 4 weeks [15]. In the United States, the Food and Drug Administration (FDA) has not yet approved any GC for use in children under the age of two years. GCs are therefore administered off-label in children under the age of two years.

In this study, we evaluated the systemic safety of gadolinium chelates with two different molecular structures and stabilities in juvenile rats. Firstly, we validated changes in renal function in juvenile rats over time. The toxicity profiles of two types of GCs with different thermodynamic and kinetic stabilities (gadodiamide and gadoteric acid) were then evaluated in juvenile rats and tissue $Gd^{3+}$ concentrations were characterized.

## 2. Materials and methods

The study protocol was approved by the in-house Animal Welfare Ethics Committee. Studies were conducted in accordance with European Directive 2010/63/EU and French legislation on animal welfare.

All studies were carried out on Sprague-Dawley rats obtained from Charles River (Charles River Laboratories, L'Arbresle, France). Animals were born at Charles River and were delivered in litters of 10 rats (five males and five females) at postnatal day (PND) 2. Animals were not weaned during the studies.

### 2.1. Characterization of renal function in juvenile rats

Animals were sacrificed at nine different timepoints: PND 4, 8, 11, 14, 16, 18, 22, 28 and 30. At each timepoint, 3 males and 3 females were sacrificed in order to detect a possible sex-linked effect. At PND 4 and 8, animals were anesthetized by intraperitoneal administration of a mixture of ketamine (95 mg/kg) and xylazine (10 mg/kg). The volume of mixture injected was calculated with a ratio of 0.2 mL/100 g of body weight. From PND 11, animals were anesthetized by isoflurane (5%) supplemented with 1 L/min $O_2$ and were then sacrificed by exsanguination by intracardiac puncture using a 1 mL heparinized syringe (up to PND 22). From PND 28, exsanguination was performed from the abdominal aorta, after laparotomy.

#### 2.1.1. Biochemistry

Plasma creatinine levels were assayed by an enzymatic method (Vitros Fusion 5.1, Ortho-Clinical Diagnostics, Inc., Issyles-Moulineaux, France) and plasma cystatin C was assayed by an immunoenzymatic method (enzyme-linked immunosorbent assay, ELISA) (Quantikine® ELISA – mouse/rat cystatin C immunoassay – R&D Systems Europe, Lille, France).

#### 2.1.2. Histology

At sacrifice, the kidneys were removed and fixed in 4% neutral buffered formalin. After dehydration, samples were paraffin-embedded, sectioned (5 μm thickness) and stained with hematoxylin-eosin-saffron (HES). Kidney samples from rats sacrificed at PND 4, PND 11 and PND 30 were examined under blinded conditions by a certified pathologist.

### 2.2. Comparative study of saline, gadoteric acid and gadodiamide in juvenile rats

Two studies were conducted: one product per litter was administered in Study 1 and three products were administered to the same litter in Study 2.

In both studies, two GCs were tested versus a control group (saline). Rats were therefore randomized to receive intravenous injections of 2.5 mmol Gd/kg (5.0 mL/kg) of gadoteric acid (Dotarem®, Guerbet, Villepinte, France), gadodiamide (Omniscan®, GE Healthcare, Chalfont St Giles, United Kingdom) or 5.0 mL/kg of isotonic saline (Lavoisier, Paris, France) on PND 4, 8, 11, 14 and 18 (cumulative dose: 12.5 mmol Gd/kg). Animals were placed in a ventilated chamber heated to 38 °C to maintain a temperature similar to that of the dam before administration. Products were injected into the jugular vein for the first two injections (on PND 4 and 8) and the caudal vein for subsequent administrations (from PND 11) in conscious animals. Both studies were blinded (administrations, clinical examinations and assays). Animals were identified by an ear-notch system and were monitored daily. Clinical follow-up was evaluated on a previously developed rating scale including behavioral signs (rejection by the dam); not eating or drinking: dehydration with persistent skinfold; motor activity: prostration, attention, immobilization, little or no locomotor activity; behavioral responses to external stimuli: reaction after stimulation, curiosity); physiologic signs (body weight, tibial length, breathing, skin lesions, digestive problems, opening of eyes, presence of chromodacryorrhea and any other abnormal sign). In both studies, the animals were euthanized at PND 25 under isoflurane

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