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European Journal of Radiology 66 (2008) 175–179

EJR
EUROPEAN JOURNAL OF RADIOLOGY

www.elsevier.com/locate/ejrad

Extracellular gadolinium contrast agents: Differences in stability

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Received 4 January 2008; received in revised form 4 January 2008; accepted 8 January 2008

Abstract

Extracellular gadolinium contrast agents (Gd-CA) are either linear or macrocyclic chelates available as ionic or non-ionic preparations. The molecular structure whether cyclic or linear and ionicity determines the stability of Gd-CA. Linear chelates are flexible open chains which do not offer a strong binding to Gd^{3+}. In contrast, the macrocyclic chelates offer a strong binding to Gd^{3+} by the virtue of being preorganized rigid rings of almost optimal size to cage the gadolinium atom. Non-ionic preparations are also less stable in comparison to the ionic ones as the binding between Gd^{3+} with the negatively charged carboxyl groups is stronger in comparison to that with amides or alcohol in the non-ionic preparations. According to stability constants and kinetic measurements, the most stable Gd-CM is the ionic-macrocyclic chelate Gd-DOTA and the least stable agents are the non-ionic linear chelates gadodiamide and gadoversetamide. In vivo data confirmed the low stability of non-ionic linear chelates but no significant difference was observed amongst the macrocyclic agents whether ionic (Gd-DOTA) or non-ionic such as Gd-HP-DO3A and Gd-BT-DO3A. The stability of Gd-CA seems to be an important factor in the pathogenesis of the serious complication of nephrogenic systemic fibrosis. Gd-CA of low stability are likely to undergo transmetallation and release free Gd ions that deposit in tissue and attract circulating fibrocytes to initiate the process of fibrosis. No cases of NSF have been observed so far after the exclusive use of the stable macrocyclic Gd-CA.

Keywords: Nephrogenic systemic fibrosis; Gadolinium chelates; Stability; Transmetallation; Chemical structure

1. Introduction

Extracellular gadolinium based MRI contrast media are all chelates containing Gd ion (Gd^{3+}). Free gadolinium is highly toxic and can cause splenic degeneration, central lobular necrosis of the liver, enzymes inhibition, calcium channels blocking and a variety of haematological abnormalities [1,2]. Therefore, it is crucially important that Gd^{3+} should be strongly attached to a chelate to avoid its toxic effects.

There are seven extracellular gadolinium contrast agents (Gd-CA) currently available for clinical use (Table 1) [3,4]. The configuration of the molecule is either linear or cyclic and they are available as ionic or non-ionic preparations. There are differences in the chemical stability of these agents and liability to release free gadolinium ions. Recently, there is increasing evidence that the instability of Gd-CA could be an important factor in the pathogenesis of the serious complication of nephrogenic systemic fibrosis (NSF) [4].

In this article, the chemical structure of Gd-CA is discussed highlighting the important features that determine the stability of the molecule. Methods to assess the stability of these agents are presented. The relevance of the stability of Gd-CA to the development of NSF is addressed.

2. Chemistry of Gd-CA

The chemical principles involved in the production of Gd-chelates are presented in a simplified manner and hopefully without important compromise of the scientific accuracy. The gadolinium ion has nine coordination sites {coordination sites represent the number of atoms or ligands directly bonded to the metal centre such as Gd^{3+}. A ligand is a molecule or atom that is bonded directly to a metal centre. The bonding between the metal centre (Gd^{3+}) and the ligands is through valent bonds in which shared electron pairs donated to the metal ion by the ligand}. In the ionic linear molecule such as Gd-DTPA (Magnevist, Bayer Schering Pharma AG, Berlin, Germany), Gd^{3+} is coordinated with five carboxyl groups and three amino nitrogen atoms. The remaining vacant site is coordinated with a water molecule, which is important in enhancing the signal by the

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doi:10.1016/j.ejrad.2008.01.025

Table 1
Stability measurements of clinically available extracellular gadolinium based contrast agents [3,4,9]

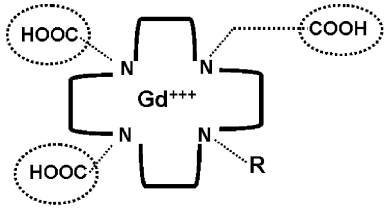
Extracellular Gd-CA	Type	Thermodynamic stability constant	Conditional stability	Amount of excess chelate (mg/ml)	Kinetic stability (dissociation half life at pH 1.0)
Gadoversetamide, Gd-DTPA-BMEA (OptiMark, Covidien, St. Louis, USA)	Non-ionic linear	16.6	15	28.4	Not available
Gadodiamide, Gd-DTPA-BMA (Omniscan, GE Healthcare, Chalfont St. Giles, UK)	Non-ionic linear	16.9	14.9	12	35 s
Gadobutrol, Gd-BT-DO3A (Gadovist, Bayer Schering, Berlin, Germany)	Non-ionic cyclic	21.8	Not available	Not available	24 h
Gadoteridol, Gd-HP-DO3A (ProHance, Bracco, Milan, Italy)	Non-ionic cyclic	23.8	17.1	0.23	3 h
Gadopentetate Gd-DTPA (Magnavist, Bayer Schering, Berlin, Germany)	Ionic linear	22.1	18.1	0.4	10 min
Gadobenate, Gd-BOPTA, (Multihance, Bracco, Milan, Italy)	Ionic linear	22.6	18.4	None	Not available
Gadoterate, Gd-DOTA (Dotarem, Guerbet, Paris, France)	Ionic cyclic	25.8	18.8	None	>1 month

contrast agent in T1 weighted MR imaging [1,2,4]. The three negatively charged carboxyl groups are neutralising the three positive charges of the Gd ion and the remaining two carboxyl groups are neutralized by two meglumine cations [1]. In the non-ionic linear molecule such as gadodiamide (Omniscan, GE Healthcare, Chalfont St. Giles, UK) and gadoversetamide (OptiMark, Covidien, St. Louis, USA) the number of carboxyl groups are reduced to three as the other two carboxyl groups, each has been replaced by a non-ionic methyl amide [1]. Although, both amide carbonyl atoms are directly coordinated to Gd^{3+} the binding is weaker in comparison to that of carboxyl groups [1,4,5]. This results in weakening the grip of the non-ionic chelate on the Gd^{3+} and decreasing the stability of the complex [4,5].

The other feature which influences the binding between the Gd^{3+} and the chelate is the configuration of the molecule whether it is cyclic or linear. The macrocyclic molecule offers a better protection and binding to Gd^{3+} by the virtue of being preorganized rigid ring of almost optimal size to cage the Gd ion. In contrast, the linear structure which is a flexible open chain offers a weaker protection of the Gd ion [4,5]. All the macrocyclic agents available for clinical use whether ionic or non-ionic are derived from 12-membered macrocyclic polyaminocarboxylates ring [5]. The number and identity of the side chains affect the stability of these agents and a minimum of three carboxylate side groups are necessary to form reasonably stable Gd-complexes (Fig. 1). Due to charge neutralization in the complexation process, lanthanides prefer carboxylate donor atoms rather than etherial or alcoholic oxygens [6,7]. The negatively charged carboxylate oxygens are more powerful donor atoms than are uncharged hydroxyl oxygen atoms [6]. Using the ionic-macrocyclic complex Gd-DOTA (Doatrem, Guerbet, Paris, France) which has four carboxylate side groups as a reference, when one carboxylate group is replaced with a hydroxypropyl group [Gd-DOTA to Gd-HP-DO3A (ProHance, Bracco, Milan, Italy)] the stability and binding constants decrease [5,6]. The replacement of sterically uncrowded hydroxypropyl group of Gd-HP-DO3A with the bulky 2,3-dihydroxy-(1-hydroxymethyl)-propyl group to form Gd-BT-DO3A (Gadovist, Bayer Schering Pharma AG, Berlin, Germany) results in further destabilization of the complex. The bulky side chain destabilizes binding interaction between Gd^{3+} and each of the three carboxylate side arms [7]. The bulky chain is also more acidic than the hydroxypropyl group and weakens the binding with Gd^{3+}. Lanthanides such as Gd^{3+} behave like typical "hard" acids and interact preferentially with hard bases than with softer bases [5,7]. Therefore, increasing the acidity of the side chain decreases the stability of the Gd-chelate [5,7]. Thus, according to these chemical principles the stability of the three available macrocyclic agents follows the order of DOTA > HP-DO3A > BT-DO3A [4]. However, it is fair to indicate that all macrocyclic agents available for clinical use are quite stable in comparison to Gd-linear chelates. For the Gd^{3+} to break free from a macrocyclic chelate it must simultaneously breaks five to six coordination sites. On the other hand, Gd^{3+} can break free easily from the linear chelate as the separation occurs sequentially [8].

2.1. *In vitro measurements to assess stability of Gd-CA*

The following measurements are used to assess the stability of the chelate molecules: thermodynamic stability constant (measured under very basic condition (pH ~11), at this pH, there



Gd-DOTA (Dotarem) **R= COOH**
Gd-HP-DO3A (ProHance) **R= Hydroxypropyl**
Gd-BT-DO3A (Gadovist) **R= Dihydroxyprpyl**

Fig. 1. The chemical structure of macrocyclic Gd-CA. All the macrocyclic agents available for clinical use have three carboxylate side groups (COOH). The difference between these agents is related to the fourth side chain (R).

are no competing hydrogen ions for the chelate and a theoretical maximum stability for the chelate is obtained); conditional stability constant (measured at physiological pH of 7.4); kinetic stability constant (dissociation half life under very acidic condition (pH 1)) [8]. The details of how to obtain these measurements are beyond the scope of this article. The higher the value of these measurements the higher is the stability of the molecule [1–4].

The amount of excess chelate in the Gd-CA preparations is another marker of the stability of these agents. A large amount of excess chelate is present in Gd-CA of low stability [3,4,9]. The excess chelate is included in the preparation to ensure absence of free Gd^{3+} in solution. The addition of excess chelate to gadodiamide (non-ionic linear chelate) dramatically reduced the acute toxicity of non-formulated preparations (no excess chelate) by a factor of 2.5 as demonstrated by acute toxicity studies (intravenous LD_{50}) [3].

As expected from the chemical structure, the stability measurements confirm the superior stability of the ionic-macrocyclic chelate and the low stability of the non-ionic linear chelates [4]. The former has the highest stability values and the longest dissociation half life and no excess chelate is required in the commercial preparation (Table 1). In contrast, the non-ionic linear chelates have short dissociation half life, the lowest stability values and the highest amount of excess chelate (Table 1) [3,4,9].

2.2. *In vivo measurements to assess stability of Gd-CA*

There are several factors in vivo such as endogenous ions, enzymes and other biological elements that may work simultaneously to dissociate the Gd-chelate with unpredictable effects [10]. Therefore, it has been suggested that ex vivo data are not reliable to predict the behavior of Gd-CA in vivo as the conditions under which these measurements are obtained are disparate from those found in vivo [10]. However, dissociation half life under acidic condition has been accepted as a reliable ex vivo measurement that can predict the stability of these agents in vivo [11].

Retention of Gd in tissues has been used to assess the stability of Gd-CA in vivo. Once the Gd cation is dissociated from the chelate, it is immediately carried away by endogenous anions such as citrates and phosphates and deposit into the body tissues. Once within the tissues, it can persist for long period of time. On the other hand, virtually all the injected chelate would be eliminated from the body by 5 days after the administration. Therefore, most of the Gd detected in the body 3–8 days after administration of a Gd-CA is likely to be released from the original chelate [8].

Thus, the higher the retention of Gd in tissues the lower is the stability of the Gd-CA.

Animal studies in vivo in rats and mice with normal renal function showed that Gd retention in tissues 2 weeks after injection of the non-ionic linear chelate gadodiamide was three times more in comparison to that observed with the ionic linear chelate Gd-DTPA. Gd retention in tissues was minimal with the tested macrocyclic agents Gd-DOTA and Gd-HP-DO3A and the least retention was observed with the non-ionic macrocyclic agent Gd-HP-DO3A [11,12]. Clinical studies have also showed that

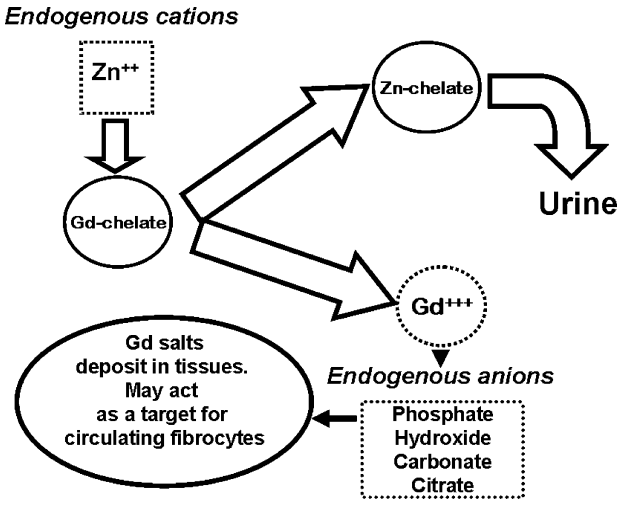


Fig. 2. A diagram of the process of transmetallation between Gd^{3+} and endogenous cations such as zinc (Zn^{2+}). The zinc replaces the Gd^{3+} of the chelate and is eliminated from the body in urine as zinc chelate. The Gd^{3+} combines with endogenous anions and deposit in tissues.

gadodiamide leaves two to four times more Gd^{3+} in the bone than Gd-HP-DO3A in patients with normal renal function [8,13].

In summary, in vivo data have confirmed the ex vivo measurements which indicate that the least stable agents are the non-ionic linear chelates. However, they did not identify any difference in the stability between the ionic and non-ionic macrocyclic agents although in vitro data suggest that the ionic-macrocyclic agent Gd-DOTA is the most stable Gd-CA. Further studies in vivo using suitable animal models of chronic renal impairment are required to elucidate whether the differences in the stability of these agents are important in vivo under biological conditions similar to those of patients with advanced reduction in renal function.

2.3. *Transmetallation*

Transmetallation of Gd-CA leads to release of free gadolinium through replacement of the Gd^{3+} within the chelate molecule by body cations such as iron, copper, zinc and calcium [14]. Only zinc can displace significant amount of Gd^{3+} because its concentration in the blood is relatively high (55–125 μmol/L), whereas copper is present in very small amount (1–10 μmol/L) and calcium ions have low affinity to organic ligands [14]. Iron ions are tightly bound by the storage proteins ferritin and haemosiderin and are not available for transmettalation with Gd^{3+} [15]. Transmettalation between Gd^{3+} and zinc will result in the formation of zinc chelate which is excreted in urine. The released Gd^{3+} becomes attached to endogenous anions such as phosphate, citrate, hydroxide or carbonate and deposit in tissues as insoluble compounds (Fig. 2) [8]. In vivo [16], in vitro [14,17] and human studies [18,19] have shown that linear chelates particularly the non-ionic ones cause a large increase in zinc excretion in urine. The non-ionic linear chelate gadodiamide induced a decrease of 32% of plasma zinc after a single injection in healthy volunteers [19]. This is thought to be secondary to transmetallation and presence of excess chelate in the gado-

diamide preparation. In patients undergoing contrast enhanced MRI examination, gadodiamide caused a large increase in zinc excretion that was higher by a factor of almost 3 in comparison to the zincuria induced by the ionic linear molecule Gd-DTPA [18]. On the other hand, the ionic-macrocyclic Gd-DOTA had no effect on zinc excretion [18]. Ex vivo studies have also confirmed that all macrocyclic Gd-CM are insensitive to transmetallation by zinc ions in comparison to the open-chain complexes [14,17].

It is of interest to note that animal studies in rats and monkeys demonstrated that repeat administration of high doses (3–5 mmol/kg) of non-ionic linear Gd-chelates (gadoversetamide and gadodiamide) for 28 days produced skin ulceration and testicular atrophy [3]. These lesions are similar to those described with zinc deficiency [20]. Much higher cumulative doses were required to produce similar lesions with the ionic agents Gd-DTPA and Gd-DOTA [3].

2.4. Stability of Gd-CA and nephrogenic systemic fibrosis (NSF)

Gd-CA are eliminated from the body through the kidneys and biological half life in patients with normal renal function is 1.5 h. In patients with advanced renal impairment elimination half life can be prolonged to 30 h or more [21]. Patients on haemodialysis would require three consecutive dialysis sessions over 6 days to remove 97% of the administered dose of Gd-CA from the body. Continuous ambulatory peritoneal dialysis for 20 days eliminates 69% of the injected dose of Gd-CA [22]. Transmetallation is likely to occur when the Gd-chelate remains in the body for a long period as is the case in patients with end stage renal disease including those on dialysis [4,21]. Transmetallation of Gd-CA leads to release of free gadolinium which is deposited in tissues as phosphates, carbonate, hydroxide or citrate complexes. The gadolinium salts would be engulfed by local macrophages leading to release of varieties of cytokines particularly transforming growth factor beta (TGF-β) which is a potent fibrogenic cytokine [23,24]. The cytokines would attract circulating fibrocytes which will leave the circulation and deposit in the dermis and other organs containing Gd. They mature into fibroblast leading to fibrotic changes and deposition of collagen in the affected tissues [24].

3. Conclusion

The chemical structure of Gd-CA determines the stability of these agents. The least stable Gd-CA are the non-ionic linear chelates and the most stable is the ionic-macrocyclic chelate. However, according to in vivo data all macrocyclic agents have similar high stability. Furthermore, no cases of NSF have been reported so far following the exclusive administration of any of the macrocyclic agents. The stability of the Gd-chelate is likely to be an important factor in the pathogenesis of NSF in patients with end-stage renal disease (ESRD).

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