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Gd-AAZTA-MADEC, an improved blood pool agent for DCE-MRI studies on mice on 1 T scanners

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A novel MRI blood-pool contrast agent (Gd-AAZTA-MADEC) has been compared with established blood pool agents for tumor contrast enhanced images and angiography. Synthesis, relaxometric properties, albumin binding affinity and pharmacokinetic profiles are reported. For *in vivo* studies, angiographic images and tumor contrast enhanced images were acquired on mice with benchtop 1T-MRI scanners and compared with MS-325, B22956/1 and B25716/1. The design of this contrast agent involved the elongation of the spacer between the targeting deoxycholic acid moiety and the Gd-AAZTA imaging reporting unit that drastically changed either the binding affinity to albumin (K_A(HSA) = 8.3 × 10^5 M^{-1}) and the hydration state of the Gd ion (q = 2) in comparison to the recently reported B25716/1. The very markedly high binding affinity towards mouse and human serum albumins resulted in peculiar pharmacokinetics and relaxometric properties. The NMRD profiles clearly indicated that maximum efficiency is attainable at magnetic field strength of 1 T. *In vivo* studies showed high enhancement of the vasculature and a prolonged accumulation inside tumor. The herein reported pre-clinical imaging studies show that a great benefit arises from the combination of a benchtop MRI scanner operating at 1 T and the albumin-binding Gd-AAZTA-MADEC complex, for pursuing enhanced angiography and improved characterization of tumor vascular microenvironment.

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1. Introduction

The advent of Molecular Imaging era has witnessed the introduction of Magnetic Resonance Imaging (MRI) pre-clinical scanners operating at lower magnetic field strength than the complex (and expensive) high field systems that are found in the specialized MR spectroscopy/imaging labs. The need for making MRI as one of the complementary imaging techniques for multi-modal studies has stimulated manufactory companies to consider the possibility of offering scanners based on permanent or electro-magnets and characterized by a user-friendly images acquisition procedure. It is expected that the availability of this new generation of MRI scanners will be a key-components for the spreading of *in vivo* Molecular Imaging facilities in biological departments together with compact PET/SPECT and Optical Imaging instruments.

In this context it appears likely that MRI scanners will be exploited in pre-clinical cancer research for evaluating novel cancer treatment approaches that require imaging methods able to accurately detect and characterize individual tumors. The last 25 years of MRI clinic studies has clearly demonstrated the important results that may be obtained by the use of paramagnetic Gd-complexes. Gd-based complexes have been extensively used in order to improve the contrast efficiency of the MRI modality, thus providing earlier tumor detection, staging and assessment of therapeutic response [1].

Two key applications of Gd-based contrast agents (CAs) appear of general use in the MRI study of tumor murine models, namely the visualization of the blood vessel network and the assessment of

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vascular permeability [2]. The best results for both applications have been obtained by using Gd(III) complexes able to reversibly bind to serum albumin [3–6]. The formation of supramolecular adducts between the paramagnetic complex and the serum protein has beneficial effects both on the attainable relaxation enhancement (particularly high at 0.5–1.5 T) and on the *in vivo* distribution properties. In fact, macromolecular Gd complexes have longer blood pool retention time while their reduced tumbling time (rotational correlation time, τ_R) increases their efficiency to relax water protons, in particular at magnetic field around 1 T [7,8]. Moreover, macromolecular CAs can preferentially accumulate in tumor tissue due to the hyperpermeability of tumor vasculature, resulting in effective tumor enhancement for precise cancer detection and delineation [9]. The longer circulation time of macromolecular CAs can also be exploited for characterizing tumor microvasculature with dynamic contrast enhanced (DCE) MRI technique, which allows to assess tumor vessel permeability/perfusion as well as to monitor non-invasively tumor response to anticancer treatment [10,11]. In addition, it is often recognized that CAs bigger in size are more efficient reporters on tumor permeability than small size CAs, because of the slower wash-in/wash-out characteristics [12,13]. Furthermore, the efficiency of a Gd complex to enhance the relaxation rate of water protons depends on the structure of the complex, the strength of the applied magnetic field and on the albumin binding affinity that need to be addressed, simultaneously, in order to improve its contrast efficiency [14–19].

In this study, we introduce a novel Gd-based CA as a blood pool and tumor vascular permeability agent and its properties are compared with those ones of the clinically approved MS-325 (Vasovist or Ablavar) [20], with B22956/1 [21], a blood-pool CA tested in Phase I trials and with B25716/1 [22].

2. Materials and methods

2.1. Chemistry

Commercially available reagents and solvents were purchased from Sigma–Aldrich or Alfa-Aesar and used without further purification. B22956/1 [23], B25716/1 and MS-325 were kindly provided by Bracco Imaging (Milan, Italy) and their chemical structure are shown in Fig. 1a–c. The protected bifunctional chelating agent 1 was prepared according to ref. [24] while methyl 3-aminodeoxycholate 2 was synthesized following the procedure reported in Ref. [25]. Reactions were monitored by TLC on Merck 60F254 (0.25 mm) plates. Spot detection was carried out by staining with an alkaline $KMnO_4$ solution or with the Dragendorff reagent. NMR spectra were recorded at 298 K on a Jeol Eclipse ECP300 spectrometer operating at 7.05 T; chemical shifts (δ) are given in ppm, coupling constants (J) in Hz. ESI mass spectra were recorded on ThermoFinnigan LCQ Deca XP-Plus and melting points (uncorrected) with a Stuart Scientific SMP3 apparatus. Human serum albumin and mouse serum albumin were purchased from Sigma–Aldrich.

2.2. Synthesis

The synthetic procedure leading to Gd-AAZTA-MADEC is reported in Scheme 1.

2.3. Synthesis of conjugate 3

Compound **1** [18] (14.0 g, 18.9 mmol) and methyl 3-aminodeoxycholate [19] (**2**, 8.50 g, 21.0 mmol) were dissolved in dichloromethane (100 mL). *N,N′*-Dicyclohexylcarbodiimide (4.9 g, 23.7 mmol) and 4-dimethylaminopyridine (0.46 g, 3.8 mmol) were added to the solution and stirred at room temperature overnight. The white solid precipitate was removed by filtration on a Buchner funnel and the filtrate evaporated under reduced pressure. The residue was submitted to chromatographic purification, obtaining the desired product as a white solid (13.6 g, 64%). M. p. 70 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 5.73 (d, J = 7.4 Hz, 1H), 4.11 (m, 1H), 3.92 (m, 1H), 3.60 (s, 3H), 3.57 (s, 4H), 3.17 (s, 4H), 2.93 (d, J = 14.1 Hz, 2H), 2.76–2.54 (m, 4H), 2.58 (d, J = 14.1 Hz, 2H), 2.36–0.95 (m, 43H), 1.385 (s, 18H), 1.382 (s, 18H), 0.91 (d, J = 6.4 Hz, 3H), 0.90 (s, 3H), 0.62 (s, 3H); ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ = 174.7 [C], 172.9 [C], 172.3 [C], 170.9 [C], 80.7 [C], 80.2 [C], 73.0 [CH], 65.4 [CH_2], 63.1 [C], 62.6 [CH_2], 59.3 [CH_2], 52.0 [CH_2], 51.5 [CH_3], 48.9 [CH], 48.3 [CH], 47.3 [CH], 46.5 [C], 45.1 [CH_2], 38.1 [CH], 37.6 [CH_2], 37.1 [CH_2], 35.9 [CH], 35.1 [CH], 34.7 [CH_2], 34.0 [CH_2], 33.0 [CH], 31.1 [CH_2], 31.1 [CH_2], 31.0 [CH_2], 30.6 [CH_2], 30.5 [CH_2], 29.7 [CH_2],

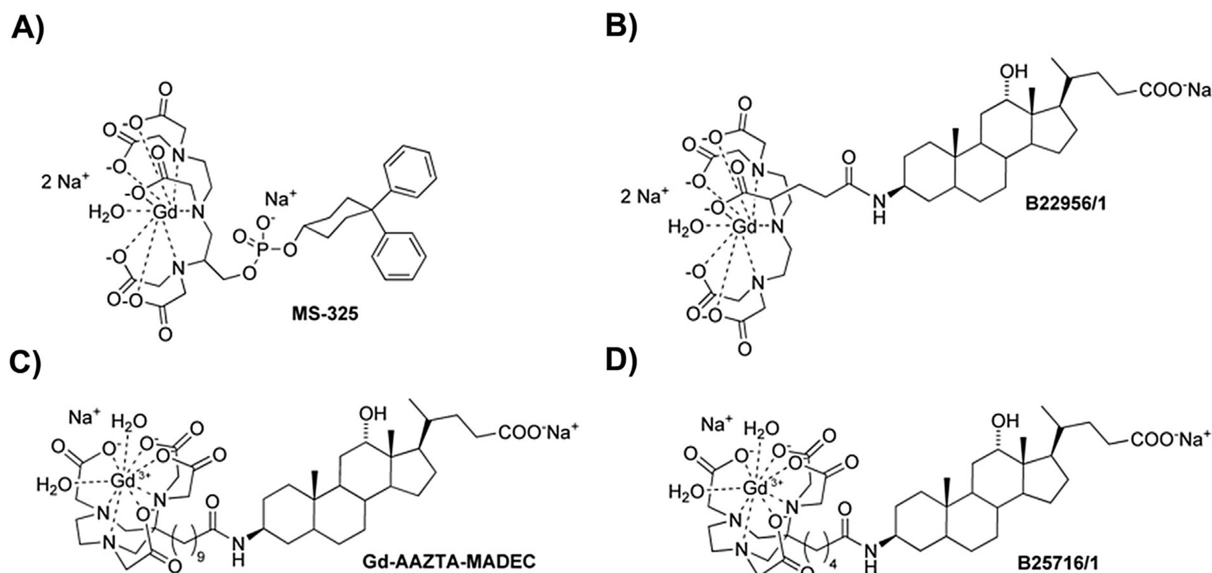

Fig. 1. Chemical structures of the investigated blood pool molecules: (A) MS-325, (B) B22956/1, (C) Gd-AAZTA-MADEC, (D) B25716/1.

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