Measurements of kinetic stability, blood-brain barrier permeability and cytotoxicity for two thulium based contrast agents

D. Coman1,2, M. Gattas-Sethi1, H. K. Trubel1,4, P. Herman5, F. Hyder2, G. Kiefer6, and F. d’Errico8

1Department of Diagnostic Radiology, Yale University, New Haven, CT, United States, 2Quantitative Neuroscience with Magnetic Resonance (QNMR), Yale University, New Haven, CT, United States, 3Bayer HealthCare Research Center, Wuppertal, Germany, 4Department of Pediatrics, HELIOS-Klinikum Wuppertal, University Witten/Herdecke, Wuppertal, Germany, 5Semmelweis University, POB 448, H-1446, Budapest, Hungary, 6Departments of Diagnostic Radiology and Biomedical Engineering, Yale University, New Haven, CT, United States, 7Macrocyclics, Dallas, TX 75235, United States

INTRODUCTION

Successful translation of smart contrast agents (SCAs) from the benchtop into biological use depends on several key intermediate steps. The measurement of ex vivo kinetic inertness, in vivo blood-brain barrier permeability and in vivo cytotoxicity are three important steps in assessing the feasibility of using any SCA for any ulcerate biological studies. Over the past decade, a new non-invasive method for simultaneous measurements of temperature and pH was developed, based on the strong dependence on temperature and pH of the proton chemical shifts from the complex between the thulium ion and the macrocyclic chelate 1,4,7,10-tetraazaacyclododecane-11-N,N,N’,-N’’,-tetra (methylene phosphonate) or TmDOTPA (Fig.1A) [1,2,3,4,5]. Thanks to the high sensitivity of each resonance on temperature and pH, models can be developed [2,3] to determine both temperature and pH simultaneously and very accurately in the rat brain [4]. More recently, a new temperature probe was introduced, also relying on thulium as paramagnetic ion, but for which the macrocyclic chelate is 1,4,7,10-tetraazaacyclododecane-1,4,7,10-tetramethyl-1,4,7,10-tetraacetate or TmDOTMA (Fig.1B) [6]. The methyl 1H chemical shift of TmDOTMA is pH-independent [6]. In the present work, we measured ex vivo kinetic inertness, in vivo blood-brain barrier permeability and in vivo cytotoxicity of TmDOTPA and TmDOTMA agents.

MATERIALS AND METHODS

In vivo animal studies: Sprague-Dawley rats were tracheotomized and artificially ventilated (70% N2O, 30% O2). During the animal preparation, isoflurane (1 to 2 %) was used for induction. An intraperitoneal line was inserted to administer α-chloralose (46 ± 4 mg/kg/hr) and an intravenous line was used to administer D-tubocurarine chloride (1 mg/kg/hr) or SCA (1-2 mmol/kg) throughout the experiment. The anesthetized rats were prepared with renal ligation as previously described [7]. All 1H NMR data (Fig. 1A, 1B and 1C) were acquired on an 11.7 T Bruker vertical-bore spectrometer (Billericia, MA). CBF was measured by a laser Doppler flowmetry probe (830 nm) and pO2 was measured by an oxygen fluorescence probe (485/600 nm).

In vivo cytotoxicity: CCL16 Chinese hamster lung cells (Fig.1D) were used to measure the in vivo cytotoxicity. The colony-forming ability of the cells in the cultures exposed to the SCAs was compared with that of cells from untreated cultures to calculate the relative surviving fraction. The cells were maintained in a humidified 95% air and 5% CO2 environment at 37 °C. After the exposure, the cultures were washed twice with Hanks’ balanced salt solution to remove debris and residual SCA, trypsinized, and counted with a Coulter counter.

RESULTS AND DISCUSSION

Kinetic stabilities of the SCAs in blood extracts from the sagittal sinuses were estimated by the intensity of their 1H NMR resonances over time. For TmDOTMA, the results indicate that there is no change in the intensity of the methyl group resonance from day 1 to day 59 (Fig.1C). Similar results were obtained for the TmDOTPA agent, indicating that the complex between the macrocyclic chelates and Tm3+ is stable over long periods of time (months). It has been proposed [6] that TmDOTPA does not cross the blood-brain barrier. Our observations suggest that TmDOTPA does cross the blood-brain barrier quite efficiently, which allows quantifying brain temperature and pH [7]. Concomitant 1H signals of TmDOTPA in blood plasma and cerebral spinal fluid (CSF) confirm that the majority of the in vivo MR signals emanate from tissue [7]. SCAs most likely enter the extracellular space despite the fact that they are charged. A plausible delivery path into the extracellular space may be through the fenestrated vessels of the circumventricular organs. The in vivo cytotoxicity tests consistently showed survival rates, expressed in terms of colony-forming ability of the exposed cells, between 90 and 95% compared to the controls (Fig.1D). Unfortunately, the half lethal dose (LD50) values of TmDOTPA or TmDOTMA in the rat have not been measured specifically, but results in our laboratory [7] suggest that an infusion dose of 1-2 mmol/kg results in stable systemic physiology without affecting normal brain function (Fig.1E). Localized changes in CBF and pO2 during forepaw stimulation in α-chloralose anesthetized rats are nearly identical before and after SCAs infusion (Fig.1E, gray and black lines, respectively). Moreover, LD50 values for similar lanthanide complexes such as YbDOTMA [8] or GdDOTA [9] are 10.5 mmol/kg and 11.4 mmol/kg, respectively, suggesting that the LD50 for the SCAs may be at least an order of magnitude higher than the doses used in our experiments. In summary, our results indicate that these two SCAs are kinetically stable, they cross the blood-brain barrier, they clearly exclude acute toxicity on Chinese hamster lung cells and they demonstrate that the brain’s activity is unaffected by their presence in the extracellular space.

REFERENCES


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