Gadolinium Complex of 1,4,7,10-tetraazacyclododecane-*N*, *N′*,*N″*,*N‴*-1,4,7-triacetic acid (DO3A) Conjugate of tranexamates: A Quest for a Liver-specific Magnetic Resonance Imaging Contrast Agent

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Introduction

Much effort has recently been made in an attempt to develop new classes of MRI CAs for use as organ targeting MRI CAs. Currently, two hepatobiliary Gd-based small molecular-weight MRI CAs, namely Gd-EOB-DTPA and Gd-BOPTA. However, these hepatobiliary Gd CAs are based on linear DTPA backbone. We have recently reported the synthesis of some gadolinium complexes of macrocyclic DO3A-tranexamates (**2a**-**b**) to find that they exhibit a hepatobiliary-specific nature. Intrigued by these observations and motivated by the recent research activities in an effort to develop multifunctional MRI CAs, we have prepared some more of DO3A-tranexamates (**1c-e**) and the corresponding Gd-complexes (**2c**-**e**) for use as a new class of liver-specific CAs. At the same time we have carried out studies on the structure activity relationship for liver-specificity.

Material and Methods

All reagents were purchased from commercial sources and used as received unless otherwise stated. DO3A were prepared according to the literature method. Characterization of new materials have been performed by analytical and various spectroscopic techniques (MRI). T_1 measurements were carried out using an inversion recovery method with variable inversion time (TI) at 1.5 T (64 MHz). T_1 relaxation times were obtained from the non-linear least square fit of the signal intensity measured at each TI value. T_2 relaxation times were obtained from the non-linear least squares fit of the mean pixel values for the multiple spin-echo measurements at each echo time (TE). Six-week-old male ICR (Institute of Cancer Research) mice with weights of 29 − 31 g were used for the MRI. The mice (n = 3) were anesthetized with 1.5 % isoflurane in oxygen. Measurements were made before and after injection of **2a-e** via tail vein. The amount of CA per each injection is 0.1 mmol [Gd]/kg for MR images. Whole body MR images were obtained with a 1.5 T MR unit (GE Healthcare, Milwaukee, WI, U.S.) equipped with a homemade small animal RF coil. The coil was of the receiver type with its inner diameter being 50 mm. The imaging parameters for SE (Spin echo) are as follows: repetition time (TR) = 300.0 ms; echo time (TE) = 13.0 ms; 8.0 mm field of view (FOV); 192×128 matrix size; 1.2 mm slice thickness; number of acquisition (NEX) = 8. Images were obtained for 24h after injection.

Results and Discussion

2a-**e** reveals R_1 relaxivities in PBS comparable well with and for some cases better than those of clinically available MRI CAs such as Dotarem® and Gadovist® (Table 1). When the comparison is made within the series, no observable differences in R_1's are perceived. Figure 2 shows the coronal T_1-weighted images of mice, ICR with Gd-DO3A conjugate of tranexamates. Of the five, the first three (**2a-c**) are comparable with Primovist® and Multihance®, typical liver-specific CAs in that they exhibit enhancement in liver for the initial 1 h. Yet, by comparison with Primovist® and Multihance®, only **2a** can be classified as a true liver-specific CA in that it shows excretion through bile. As for **2b** and **2c**, strong enhancement in liver is also notable, yet no bile-excretion is observed. The signal intensity with **2e** is as weak as that with Gadovist®, a typical ECF agent (Figure 1). Although **2d** shows initially strong enhancement both in kidney and abdominal aorta, it is to be classified as a ECF agent as well in that excretion is almost complete within an hour like Gadovist®. The presence of lipophilic ethoxybenzyl in Primovist® and Multihance® is believed to play a key role in all this process. In this regard, the presence of lipophilic cyclohexyl moiety in **2a** may also be playing a similar role. This issue is the subject of future investigation.

Conclusions

A new series of DO3A conjugates of tranxamates (**1c**-**e**) and their Gd complexes (**2c**-**e**) were prepared for use as a liver-specific MRI CA. All these complexes show thermodynamic and kinetic stabilites, R_1 relaxivities comparable to those of structurally related clinical agents such as Dotarem®. A brief study on the structure activity relationship was carried out to find that of five complexes (**2a**-**e**), only **2a** reveals liver-specificity. Although **2b** and **2c** show strong enhancement in liver, yet no bile-excretion is observed to be termed as a liver-specific agent. The rest behaves much like ordinary ECF CAs like Dotarem®. The new series possess no toxicity to be employed in vivo.

Chart 1. Gd-DO3A conjugate of tranexamates.

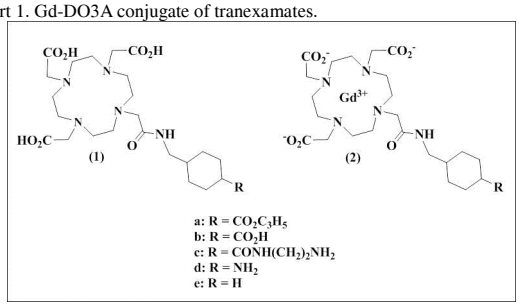

Table 1. Relaxivities of Gd-DO3A conjugate of tranexamates in PBS at the 1 mM concentrations and 293 K.

	R_1 [mM^{-1} s^{-1}]	R_2 [mM^{-1} s^{-1}]
2a	4.84 ± 0.18	4.91 ± 0.25
2b	3.87 ± 0.11	3.98 ± 0.26
2c	3.68 ± 0.13	3.62 ± 0.26
2d	3.94 ± 0.13	3.68 ± 0.29
2e	4.49 ± 0.18	3.87 ± 0.20
Dotarem®	3.59 ± 0.17	3.87 ± 0.20
Gadovist®	4.38 ± 0.15	4.27 ± 0.30

Figure 1. *In vivo* MR coronal images of mice obtained with Gd-DO3A conjugate of tranexamates. (A) The coronal T_1-weihted images of ICR mice 5 min after injection with CAs: K, Kidney; A, Abdominal aorta. (B) The coronal and axial T_1-weihted images of ICR mice 1 h after injection with CAs: H, Heart; L, Liver; B, Bladder; G, Gallbladder