In vivo animal study of the excretion time of a macrocyclic Gadolinium Based Contrast Agent and the specific retention in bladder, spleen and bone

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INTRODUCTION

Gadolinium based contrast agents (GBCAs) are commonly employed at clinical settings to add relevant information to the anatomical resolution of the magnetic resonance images[1]. In recent years, concern on the use of GBCAs has raised as it has been found that tiny amount of Gd can be retained in brain and other tissues, also in patients without renal dysfunctions[2–5]. Whereas much work has been carried out to investigate the issue of Gd-retention in the brain, the aim of this work is to bring the attention to tissues less considered in the past such as bladder, spleen and bones.

METHODS

In order to evaluate the amount of Gd retained in the tissues, mice were administered with **20 doses of 0.6** mmol **Gadoteridol/kg over a period of 4 weeks.**
The sacrifice time was set at four different time points (4, 15, 30 and 90 days) after the last injection. After sacrifice, urine, tissues and organs were collected. One tibia was weighted, mineralized and Gd quantified through ICP-MS. The other tibia was handled in order to separate bone matrix and bone marrow and Gd quantified separately.
Spleen was processed as well, to measure the amount of Gd in the splenocytes and in its fibrous part. Bladder, kidney, liver and spleen were collected and fixed to perform specific staining for analysing connective tissue, collagen and extracellular matrix components.

RESULTS/DISCUSSION

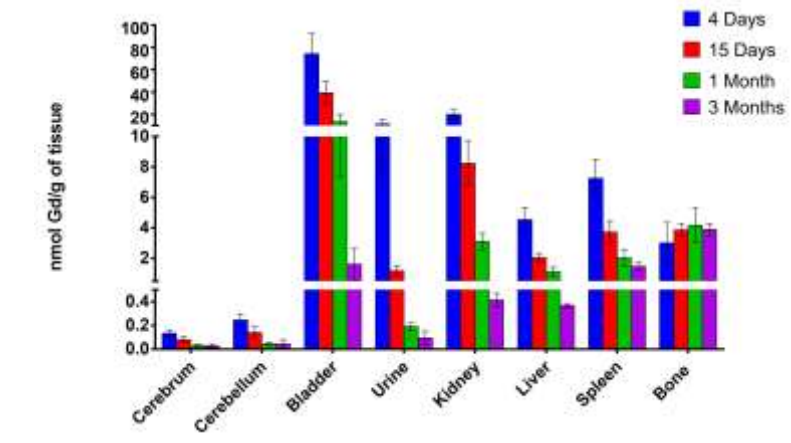

Figure 1: Amount of Gd^{3+} found in the organs of mice 4, 15, 30 and 90 days after the last of 20 injections (0.6 mmol/Kg each) of gadoteridol as determined by ICP-MS analysis.

The quantification of Gd in each collected organ and tissue at different time points shows that Gd is progressively excreted from all the investigated organs but in bones, where the amount of metal remains constant (Fig.1). However, in all the investigated organs, the amounts of residual Gd^{3+} at 3 months are significantly higher than the baseline values determined in the organs of control mice which never received GBCA injection.
The amount of Gd found in the spleen decreased in time, and the metal found in each compartment (splenocytes and fibrous part) is quite surprising. Indeed, the Gd detected in the fibrous part is greater than that found in the splenocytes (Fig. 2), however, the metal concentration in the whole spleen is even higher.
The quantitative analysis of the whole tibia shows a constant quantity of metal retained until 90 days after the last administration. The separate analysis of bone marrow and bone matrix, reveals that most of Gd is retained constantly by the bone matrix, while a very low, and time decreasing, amount of metal was found in the bone marrow (Fig. 3). Also in this case, the metal is not completely recovered, after processing the tissue.

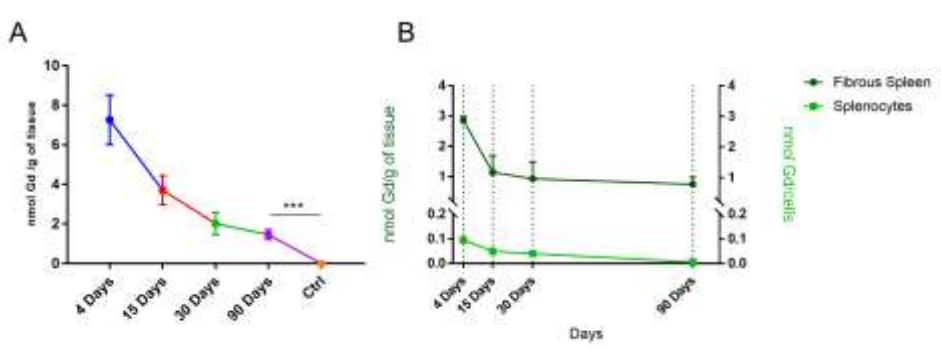

Figure 2: (A) Gadoteridol excretion curve in spleen. The yellow points correspond to the baseline values determined in control mice which never received GBCA administration. (B) Gadoteridol excretion curve in the fibrous spleen and in the splenocytes.

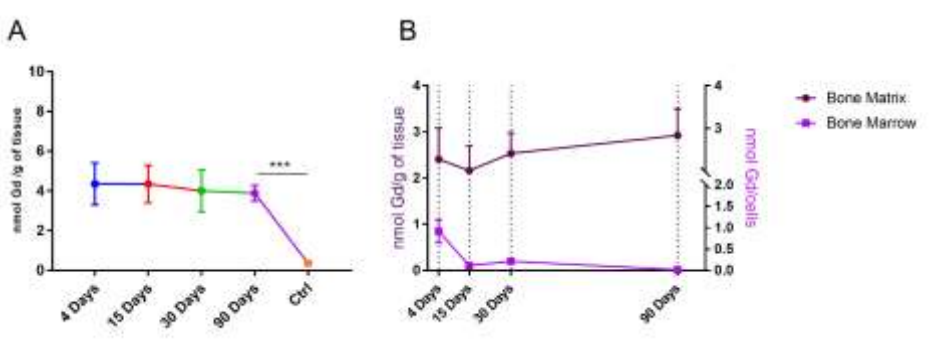

Figure 3: (A) Gadoteridol excretion curve in the whole tibia. The yellow points correspond to the baseline values determined in control mice which never received GBCA administration. (B) Gadoteridol excretion curve in bon matrix and bone marrow.

In bladder, the highest amount of metal, among all the investigated organs, is retained, especially at the shortest times. Urine samples were also analysed to determine the rate of elimination over time. Urine Gd concentration rapidly decrease over time to suggest that most of the administered GBCA is correctly excreted through the renal route (Fig. 2).

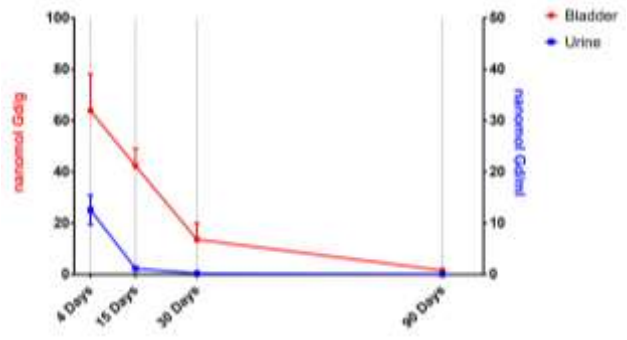

Figure 4: Gadoteridol excretion curve in bladder and in urine.

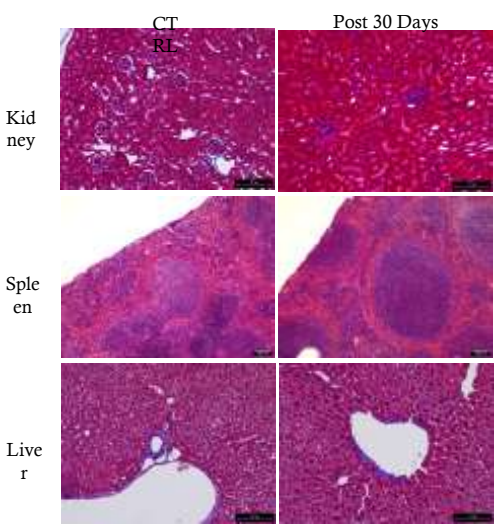

Figure 5: Trichrome staining on kidney, spleen and liver samples. he blue-colored parts highlight collagen, which in this case is almost absent at every time points

Trichrome staining for connective tissue does not show any differences with respect to control and each time points in any tissue, but bladder (Fig5). The outcomes show that, in bladder, it appears like there is more matrix at increasing time points; in addition, an hyperplasia of the urothelium could also be assessed (Fig 6).

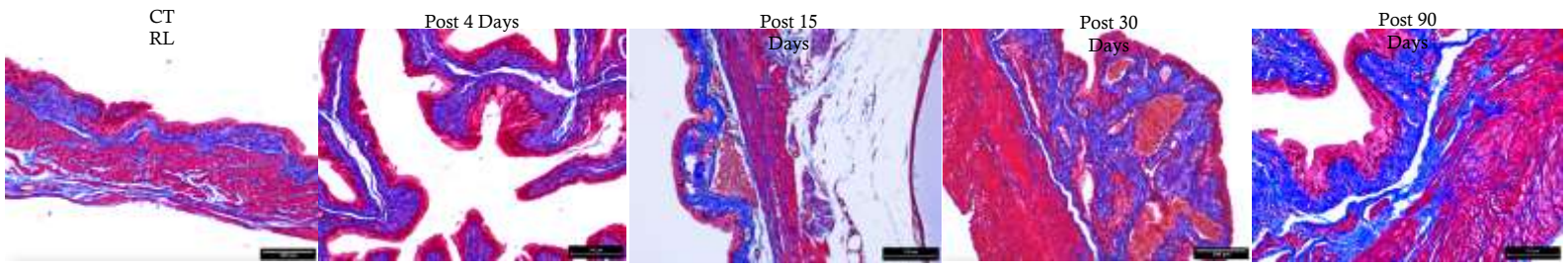

Figure 6: Trichrome staining on bladder samples. The blue-colored parts highlight collagen.

CONCLUSIONS

The outcomes point out that bladder could be an extremely specific organ for the metal retention. This result is likely related to the passage and stasis of urines, which contain a very large amount of GBCA starting from the hours immediately after the administration. In the spleen, the higher amount of Gd is found in its fibrous part: probably due to the characteristic and unique extracellular matrix[6], Gd could be stuck, after an initial washout. Indeed, the quantity of metal remains almost at the same level in both whole spleen and its fibrous part after 30 days. The analysis on bones showed a very quick deposition of Gd: as a matter of fact, the excretion rate is very low, in particular in the bone matrix, where most of Gd is retained. The amount of metal lost during the procedure to separate each component of spleen and bone is certainly something to think about, to better understand the behaviour of Gd in these organs. The trichrome staining pointed out a possible correlation between the higher concentration of Gd and the thickening of the bladder epithelium: this result must be investigated at longer time points than 90 days, to determine the possible negative long-term impact on human body.

References

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