Reversible and Irreversible Transverse Relaxation Rates of Iron-Overloaded Livers in the Thalassemic Mice

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Introduction

In iron overloading diseases such as thalassemia, excess iron levels in the body caused by repeated blood transfusions can lead to serious consequences including tissue injury, fibrosis, or even cardiac failure. Accurate determination of iron levels could therefore greatly benefit the prognosis and help monitor effects of treatment. Currently, the SQUID technique is the only established non-invasive technique for accurate iron quantification (1-2). Its limited availability and high cost, however, preclude its widespread clinical use. MRI offers an alternative for estimating iron content non-invasively. Although the relaxation rates R_2 (3-5) and R_2* (6) both have separately been shown to increase with higher iron levels, the relative contributions of the irreversible (R_2) and reversible ($R_2' = R_2^* - R_2$) relaxation rates are currently unknown. In this work, the relaxation parameters R_2*, R_2, and R_2' are analyzed using the GESFIDE (gradient-echo sampling of free induction decay and echo) sequence (7) in a mouse model of thalassemia. It is shown in this preliminary study that in iron-overloaded murine liver specimen, the three relaxation rates are all positively correlated with iron concentration, and that contribution from R_2' to the total relaxation R_2* is increasingly greater at elevated iron levels.

Methods

The Mouse Model: A thalassemia mouse model was chosen in this study due to the similarity of the disease to the human counterpart. Heterozygous β-thalassemic mice were created by mating transgenic sickle mice with wild type mice. (Homozygotes die in utero.) In order to elevate the iron levels in the thalassemic mice to levels greater than the typical two-fold increase, a sub-group of these mice were peritoneally transfused with blood from other mice (approx. 0.5 cc, twice a week for approximately 8 weeks) as a means to induce greater iron overload.

Scan Protocol: In the GESFIDE technique, a train of gradient echoes is acquired following the excitation pulse and a second set of echoes following the 180° refocusing pulse (7). When fit to a decaying exponential, the echoes prior to the 180° yield R_2^* ($= R_2 + R_2'$), while those following evolve with a rate constant R_2^- ($= R_2 - R_2'$). R_2 and R_2' could subsequently be computed by a linear combination. The GESFIDE sequence was implemented on a 1.5T Siemens Sonata MR scanner. A total of 13 mice were used in this study (5 wild type, 5 thalassemic, 3 thalassemic with transfusion). Following cervical dislocation, the liver samples were harvested and individually inserted into plastic vials filled with saline. The tubes were oriented parallel to the magnetic field to minimize field perturbations. A single axial slice was prescribed with the following parameters: 5 echoes per echo train, 6.6 ms inter-echo spacing, TR = 500 ms, 3 mm slice thickness, 10 cm FOV, matrix size = 256x256, receiver bandwidth = ±60kHz, 1 or 2 averages. R_2^* and R_2^- maps were computed on a pixel-by-pixel basis from the two echo trains, and R_2 and R_2' computed by subsequent linear combination.

Results and Discussion

The average relaxation rates for the three groups of mice are shown in **Table 1**, showing substantial differences among the three groups. R_2*, R_2 and R_2' are all increased in the diseased livers, particularly in the transfused group, with R_2' affording the greatest relative increase. Thus the relative contribution of R_2' to the total relaxation (R_2*) increases with iron concentration. The plot of relaxation rates vs. iron concentration are shown in **Fig. 1**, demonstrating the positive correlation of all three parameters with iron content. The correlation coefficients *r* were 0.62, 0.57, and 0.64 for R_2*, R_2 and R_2', respectively.

The GESFIDE technique allows the separation of the reversible and irreversible components of the transverse relaxation, which may be useful for differentiating the form in which the iron is stored, as large clustering of iron particles in hemosiderin in high iron-overload may cause greater enhancing effect on R_2' than on R_2 [8]. The small range of iron concentrations observed in the murine livers (approx. a factor of 3 - 4 in this study) is likely the reason for the lower correlation coefficients observed in the current study compared to those of R_2* from studies in human thalassemic subjects ($r \sim 0.8$), where the increase in iron concentration can be 2 orders of magnitude or greater. The small specimen size also increases the likelihood of partial voluming, which may artifactually reduce the measured relaxation rates due to the surrounding saline solution.

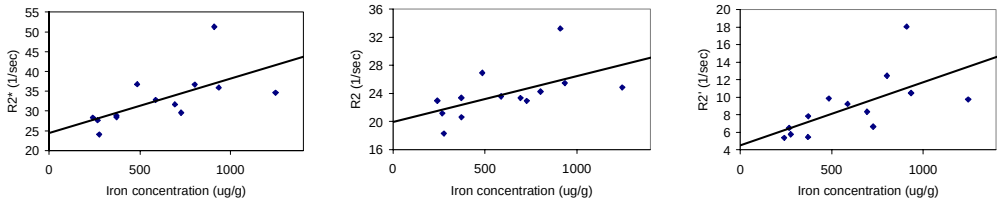

Fig. 1 Relaxation rates vs. iron concentration for the murine liver specimen.

Conclusion

In this preliminary work, it is demonstrated that the MR relaxation parameters R_2*, R_2 and R_2' are all well correlated with murine liver iron content. Within the limited range of iron concentrations investigated, the relative change in R_2' is greatest among the three. Thus, the relative contribution of the reversible component R_2' to the total relaxation R_2* increases with iron concentration. The GESFIDE sequence was capable of measuring these parameters efficiently in a single scan.

Table 1 Average iron content (μg/g) and relaxation rates (s^{-1}).

	Wild-type	Thalassemia	Thalassemia Transfused
[Fe]	305 ± 96	658 ± 124	1030 ± 188
R_2*	27.5 ± 4.6	33.5 ± 3.2	40.6 ± 9.3
R_2	21.3 ± 3.2	24.2 ± 1.6	27.9 ± 4.7
R_2'	6.2 ± 2.0	9.3 ± 2.1	12.8 ± 4.6

Acknowledgement: R01-DK-066129

References

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