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BRIEF COMMUNICATION

Rivastigmine decreases brain damage in HIV patients with mild cognitive deficits

Gaetano Perrotta[1,a], Guillaume Bonnier[2,a], Djalel-Eddine Meskaldji[3,4,5], David Romascano[6], Ruslan Aydarkhanov[7], Alessandro Daducci[6], Samanta Simioni[1], Matthias Cavassini[8], Melanie Metral[1], François Lazeyras[9], Reto Meuli[10], Gunnar Krueger[11], Renaud A. Du Pasquier[1] & Cristina Granziera[1,2]

[1]Department of Clinical Neurosciences, Service of Neurology, Neuroimmunology Unit, Lausanne University Hospital and University of Lausanne, Lausanne, Vaud, Switzerland
[2]A.A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, USA
[3]Institute of Bioengineering, École Polytechnique Fédérale de Lausanne, Lausanne, Vaud, Switzerland
[4]Department of Radiology and Medical Informatics, University of Geneva, Geneva, Switzerland
[5]Applied Statistics, Institute of Mathematics, École Polytechnique Fédérale de Lausanne, Lausanne, Vaud, Switzerland
[6]Signal Processing Laboratory (LTS5), École Polytechnique Fédérale de Lausanne, Lausanne, Vaud, Switzerland
[7]Advanced Clinical Imaging Technology, EPFL, Lausanne, Vaud, Switzerland
[8]Department of Infectious Diseases, Lausanne University Hospital and University of Lausanne, Lausanne, Vaud, Switzerland
[9]Department of Radiology, Geneva University Hospital and University of Geneva, Geneva, Switzerland
[10]Department of Radiology, Lausanne University Hospital and University of Lausanne, Lausanne, Vaud, Switzerland
[11]Siemens Healthcare, Malvern, Pennsylvania
[a]contributed equally to this study

Correspondence
Cristina Granziera, Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA. Tel: +1 617-643-4883; Fax: +1 617-726-7422;
E-mail: cgranziera@mgh.harvard.edu

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Abstract

Rivastigmine has been shown to improve cognition in HIV+ patients with minor neurocognitive disorders; however, the mechanisms underlying such beneficial effect are currently unknown. To assess whether rivastigmine therapy is associated with decreased brain inflammation and damage, we performed T1/T2* relaxometry and magnetization transfer imaging in 17 aviremic HIV+ patients with minor neurocognitive disorders enrolled on a crossed over randomized rivastigmine trial. Rivastigmine therapy was associated with changes in MRI metrics indicating a decrease in brain water content (i.e., edema reabsorption) and/or reduced demyelination/axonal damage. Furthermore, MRI changes correlated with cognitive improvement on rivastigmine therapy.

Introduction

Rivastigmine is a dual acetyl- and butyrylcholinesterase inhibitor (ChEI) that is commonly used to alleviate cognitive deficits in Alzheimer's disease (AD).[1] Recently, we have provided first evidence that rivastigmine improves some cognitive deficits in HIV patients with mild neurocognitive disorder (MND).[2] In AD patients,

rivastigmine achieves its positive effects on cognition through multiple mechanisms encompassing enhanced cholinergic neurotransmission and improved clearance of amyloid plaques.[3] The putative beneficial effect of rivastigmine in HIV+ patients may be due to a decrease in brain acetylcholine levels, related to a deficit in choline acetyltransferase.[4] Yet, in HIV patients, rivastigmine may also improve cognitive outcome by reducing brain inflammation and damage. Indeed, rivastigmine has been reported to markedly temper microglia activation, demyelination, and axonal damage in experimental autoimmune encephalomyelitis, leading to improved spatial memory deficits.[5] Also, in a cellular model of HIV-associated dementia, the adjunction of galantamine, another ChEI, resulted in the inhibition of microglial activation induced by HIV-1 proteins.[6] Moreover, the activation of the cholinergic anti-inflammatory system by nicotine was shown to attenuate neuroinflammation via suppression of Th1 and Th17 responses.[7]

In this study, we used multiparametric MRI (mpMRI) to investigate the effect of rivastigmine on brain inflammation and damage in MND HIV patients who participated to a blinded randomized crossed over trial to assess rivastigmine efficacy and safety.[2] Multiparametric quantitative MRI represents a sensitive approach to study inflammatory and degenerative brain changes.[8] In fact, quantitative MRI metrics such as T1 and T2* relaxometry and semiquantitative MRI parameters like magnetization transfer ratio (MTR) may provide complementary information regarding the brain tissue structure and its components: T1 relaxation times are sensitive to the tissue organization of micro- and macromolecules, and to a lesser extent to iron content;[9] T2* relaxation times exhibit high sensitivity to quantify paramagnetic substances like iron and provide additional information concerning the presence of free and bound water molecules (i.e., myelin water[9]). On the other hand, MTR is highly sensitive to the presence and extent of macromolecules such as myelin, cellular membranes components, etc.[9]

Therefore, the combination of T1/T2 relaxometry and MTR may help disentangling whether brain structural changes underlie the benefits of rivastigmine in HIV patients.

Methods

Subjects' population

We studied 17 patients with HIV infection, undetectable viremia, and MND, who were enrolled in a blinded crossover randomized study to assess efficacy and safety of rivastigmine for the treatment of MND.[2] MND was diagnosed according to Frascati criteria[10] using an extensive neuropsychological and neuropsychiatric battery of tests.[2] Exclusion criteria were previous brain opportunistic infection, any other opportunistic infection in the last year, active drug consumption, and major depression according to *DSM-IV* criteria. No patient developed psychiatric (e.g., psychosis) or neurological conditions (e.g., stroke), which could have interfered with neuropsychological or MRI evaluation. Patients clinical characteristics are summarized in Table S1.

Patients were randomly assigned to two arms receiving either rivastigmine or placebo for 20 weeks, followed by a 6 weeks washout, and then by 20 weeks where treatments were inversed between the two arms (see Simioni et al.[2] for details).

All patients had undetectable HIV-RNA in the plasma (defined as <20 copies/mL for more than 3 months) and in the cerebrospinal fluid (<200 copies/L) at study entry; at study end, plasmatic HIV-RNA was detected in one patient (147 copies/mL).

Brain MRI scans were performed before the treatment was started (time point 0, TP0), at 20 weeks (TP1), and at 46 weeks (TP2) (Fig. 1). Five patients could not complete the MRI follow-up and were therefore excluded from the analysis.

A neuropsychological evaluation was also performed at TP0, TP1, and TP2 for all subjects. Neuropsychological tests explored cognitive domains which are usually impaired in MND patients. They included reaction time and rapid visual information processing and spatial working memory (strategy and error component) from the Cambridge Neuropsychological Test Automated Battery (CANTAB), Trail Making Test Parts A and B, Wechsler Adult Intelligence Scale-III Symbol Digit Test, CANTAB, Digit Spans Backward/Forward, and Stockings of Cambridge (see 2 for details).

Magnetic Resonance Imaging

All MRI scans were performed on a 3T Magnetom Trio (a Tim System, Siemens, Erlangen, Germany) equipped with a 32-channel head coil.

The study protocol consisted a high-resolution, T1-weighted magnetization prepared rapid gradient echo

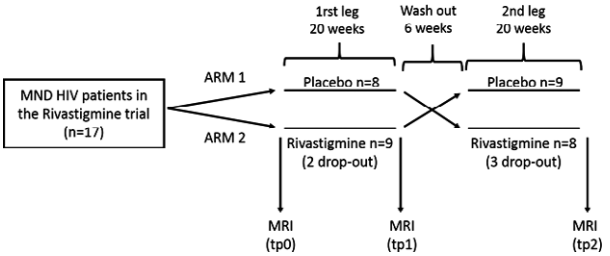

Figure 1. Study design and MRI time points (TP).

(MPRAGE) acquisition for anatomical reference; an MP2RAGE acquisition with the same voxel and matrix size to obtain T1 relaxation maps;[11] and a multiple echo Fast Low Angle SHot (FLASH) with and without magnetization transfer preparation. The signals acquired with (MT) and without (M0) magnetization saturation pulse were used to compute magnetization transfer ratio (MTR) maps as reported previously.[8,12] T2 star maps were calculated using a monoexponential fitting as performed previously.[8,12] Brain segmentation into gray and white matter (GM and WM) was performed using Morphobox[13] (see Data S1) and segmentation quality was visually inspected. Mean T1 rt, T2* rt, and MTR were calculated in each ROI (WM and GM) at each time point. The study was approved by the ethics committee of the *Centre Hospitalier Universitaire Vaudois* (CHUV) in Lausanne (Switzerland), and all subjects gave informed written consent.

Statistical analysis

Analysis of variance of multiparametric brain MRI data

To measure the effect of rivastigmine on brain microstructural properties, we computed for each subject the difference in T1rt, T2* rt, and MTR between TP1 and TP0 and TP2 and TP1 in WM and GM. Subsequently, we performed a multivariate analysis of variance (MANOVA) for each MR contrast and each brain tissue. Treatment and sequence of treatment (Arm1, Arm2) were considered as fixed effects. Bonferroni correction for multiple comparison was applied (6 MANOVA; 2 regions of interest, that is, WM and GM; and 3 MR parameters, that is, T1 rt, T2*rt, and MTR).

Our null hypothesis was that there is no difference in parametric MRI between patients treated with rivastigmine and patients who received placebo.

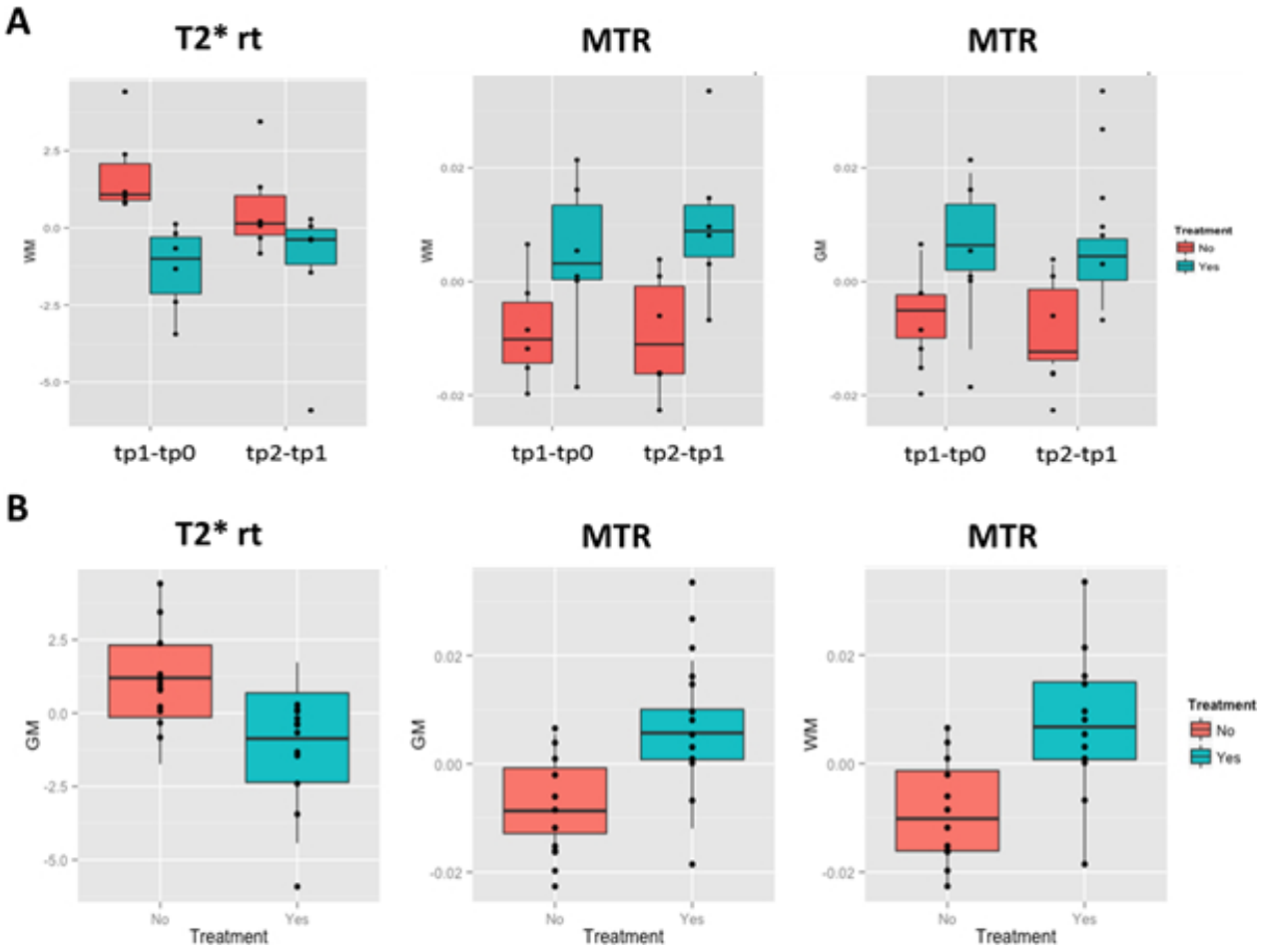

Figure 2. (A) Boxplot with superimposed dotplot showing significant differences ($P < 0.01$ and corrected-$P < 0.05$) obtained using crossed MANOVA between rivastigmine-treated and placebo-treated patients for T2* rt in WM and MTR in WM and GM. Untreated patients in the two arms are shown in light red, treated patients in light blue. (B) Summary plot showing significant differences ($P < 0.01$ and corrected-$P < 0.05$) in T2* rt in GM and MTR in GM and WM in patients treated with rivastigmine (light blue) and placebo (red).

To investigate the relationship between changes in MRI metrics and changes in cognition during rivastigmine treatment, we used a generalized linear model (GLM) and assessed whether a linear regression of the variation of T2* and MTR in WM and MTR in GM predicted the variation in the Trail Making Test, Part A (TMTA) *z*-score (calculated using available normative data), which is the score that improved on rivastigmine in the previous pilot trial in MND patients.[2]

Results

Mean T2* rt in WM was significantly different between patients treated with rivastigmine and those treated with placebo ($P = 0.004$, P-corr = 0.02, degrees of freedom: 11, standard error: 0.69; Table S2). Patients treated with rivastigmine showed a decrease in WM T2* rt over time, while patients treated with placebo exhibited an increase in mean T2* rt (Fig. 2).

Mean MTR in WM and GM was significantly different between patients treated with rivastigmine and those treated with placebo ($P = 0.006$, P-corr = 0.04, degrees of freedom: 11, standard error: 0.004 for both; Table S2). Patients treated with rivastigmine showed an increase in MTR in both WM and GM, while patients treated with placebo exhibited a decrease in MTR (Fig. 2).

T1 rt did not show any significant difference in WM and GM between subjects with and without treatment.

The GLM analysis showed that the difference between MTR after rivastigmine treatment and before rivastigmine treatment (delta MTR) significantly correlated with the difference in TMTAZ after and before treatment (delta TMTAZ; adjusted $R^2 = 0.4$, $P = 0.03$; Fig. 3).

Discussion

Our study provides evidence that rivastigmine treatment reduces brain damage in MND HIV patients. Also, we show that changes in MRI metrics on rivastigmine were correlated with clinical improvement.

A recent pilot study performed by our group showed that rivastigmine treatment for 20 weeks leads to an improvement in processing speed and executive function in HIV subjects with minor neurocognitive disorder.[2] Still, whether brain structural changes underlay cognitive improvement on rivastigmine in HIV-infected patients is currently unknown.

Rivastigmine has been previously reported to decrease brain inflammation (i.e., microglia activation,[5] also when triggered by HIV proteins[6]) and brain damage (demyelination and axonal injury[5]) in animal models and cell cultures. We hypothesized that the cognitive improvement measured in the rivastigmine trial in HIV patients[2] is paralleled by reduced brain inflammation and reversible damage to myelin and axons.

Multiparametric MRI provides sensitive biomarkers of brain inflammation and degeneration in HIV-infected patients. By combining T1 and T2* relaxometry with magnetization transfer imaging, we have shown that aviremic HIV patients with MND exhibit increased and diffuse brain inflammation and degeneration compared to aviremic HIV patients without MND and to healthy subjects.[12] In this pilot study, we applied exactly the same mpMRI approach as described in Granziera et al.[12] to assess the effect of rivastigmine on brain inflammation and damage.

The concomitant decrease in MTR and T2* rt observed in patients treated with rivastigmine strongly suggests an increase in macromolecules (i.e., myelin or axons) and/or a decrease in free water (i.e., edema reabsorption that may induce an increase in MTR).

In fact, MTR parameter quantifies the interaction between free protons and immobilized protons bound to macromolecules and an increase in MTR has been linked to an increase in myelination/axons[14–16] or decrease in water content.[17–19] On the other hand, T2* relaxation times reflect the loss of transverse magnetization due to T2 relaxation; therefore, a decrease in T2* relaxation times may be provoked by an increase in macromolecular compounds such as myelin.[20,21] Interestingly, changes in brain

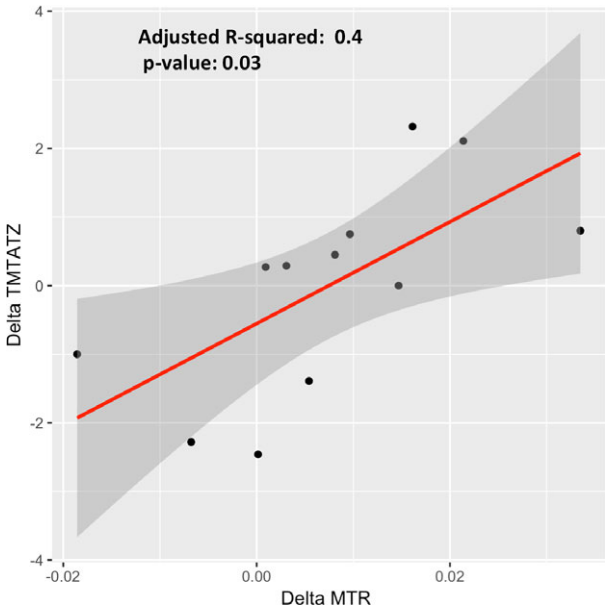

Figure 3. Correlation plot showing the positive relationship between changes in the Trail Making Test A (TMTA) – normalized using available normative data in TMTA *z*-score (TMTAZ) – and changes in MTR after treatment. Changes in TMTAZ and MTR were calculated as the difference between TMTAZ and MTR after rivastigmine treatment and a TMTAZ and MTR at baseline.

white matter MTR (i.e., increase in MTR) were significantly related to the improvement in processing speed (Trail Making Test Part A scores) on rivastigmine treatment, suggesting that the improvement in cognitive function – reported in our previous pilot trial on rivastigmine efficacy[2] – may be mediated by a decrease in brain damage.

Our study suffers from some methodological limitations. The first one is the small sample size of our cohort. The second is that the MRI at TP1 was performed just before the washout period, decreasing the sensitivity to detect changes among time points. However, we tried to take this latter effect into account by including the study arm (i.e., sequence of treatment) as covariable in our multivariate analysis.

In conclusion, we have shown that rivastigmine treatment leads to a reduction in brain damage, alongside to improved cognition in a group of HIV patients with MND. Future studies should confirm and extend our current findings in larger cohorts of patients and combining mpMRI with biological assessments of inflammatory markers in serum and cerebrospinal fluid before and after rivastigmine treatment.

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Authors' Contributions

C.G., G.K., and R.D.P. designed the study and G.P., D.-E.M., G.B., D.R., R.A., A.D., S.S., M.C., M.M., F.L., R.M., G.K., R.D.P., and C.G. collected, analyzed, and interpreted the data, wrote the report, and submitted the paper for publication.

Conflict of Interest

S. Simioni has received funding for travel or speaker honoraria from Gilead and Boehringer Ingelheim. R. Du Pasquier has served on scientific advisory boards for Biogen Idec, Merck Serono, Teva, and Novartis, and has received funding for travel or speaker honoraria from Abbott, Biogen Idec, Teva, Merck Serono, Bayer Schering Pharma, and ViiV. C. Granziera serves on scientific advisory boards for Novartis and has received speaker honoraria and travel funding from Novartis. G. Krueger is a Siemens AG employee. The other authors have nothing to report.

References

1. Huang Y, Mucke L. Alzheimer mechanisms and therapeutic strategies. Cell 2012;148:1204–1222.
2. Simioni S, Cavassini M, Annoni J-M, et al. Rivastigmine for HIV-associated neurocognitive disorders: a randomized crossover pilot study.Neurology 2013;80:553–560.
3. Onor ML, Trevisiol M, Aguglia E. Rivastigmine in the treatment of Alzheimer's disease: an update. Clin Interv Aging 2007;2:17–32.
4. Koutsilieri E, Czub S, Scheller C, et al. Brain choline acetyltransferase reduction in SIV infection. An index of early dementia? NeuroReport 2000;11:2391–2393.
5. Nizri E, Irony-Tur-Sinai M, Faranesh N, et al. Suppression of neuroinflammation and immunomodulation by the acetylcholinesterase inhibitor rivastigmine. J Neuroimmunol 2008;203:12–22.
6. Giunta B, Ehrhart J, Townsend K, et al. Galantamine and nicotine have a synergistic effect on inhibition of microglial activation induced by HIV-1 gp120. Brain Res Bull 2004;64:165–170.
7. Nizri E, Irony-Tur-Sinai M, Lory O, et al. Activation of the cholinergic anti-inflammatory system by nicotine attenuates neuroinflammation via suppression of Th1 and Th17 responses. J Immunol Baltim 1950;2009:6681–6688.
8. Bonnier G, Roche A, Romascano D, et al. Advanced MRI unravels the nature of tissue alterations in early multiple sclerosis. Ann Clin Transl Neurol 2014;1:423–432.
9. Helms G. Tissue properties from quantitative MRI in Brain Mapping: An Encyclopedic Reference Lund: Elsevier, 2015.
10. Antinori A, Arendt G, Becker JT, et al. Updated research nosology for HIV-associated neurocognitive disorders. Neurology 2007;69:1789–1799.
11. Marques JP, Kober T, Krueger G, et al. MP2RAGE, a self bias-field corrected sequence for improved segmentation and T1-mapping at high field. NeuroImage 2010;49:1271–1281.
12. Granziera C, Daducci A, Simioni S, et al. Micro-structural brain alterations in aviremic HIV+ patients with minor neurocognitive disorders: a multi-contrast study at high field. PLoS ONE 2013;8:e72547.
13. Schmitter D, Roche A, Maréchal B, et al. An evaluation of volume-based morphometry for prediction of mild cognitive impairment and Alzheimer's disease. Neuroimage Clin 2015;7:7–17.
14. Schmierer K, Scaravilli F, Altmann DR, et al. Magnetization transfer ratio and myelin in postmortem multiple sclerosis brain. Ann Neurol 2004;56:407–415.
15. Moll NM, Rietsch AM, Thomas S, et al. Multiple sclerosis normal-appearing white matter: pathology-imaging correlations. Ann Neurol 2011;70:764–773.

16. Schmierer K, Wheeler-Kingshott CAM, Tozer DJ, et al. Quantitative magnetic resonance of postmortem multiple sclerosis brain before and after fixation. Magn Reson Med 2008;59:268–277.
17. Vavasour IM, Laule C, Li DKB, et al. Is the magnetization transfer ratio a marker for myelin in multiple sclerosis? J Magn Reson Imaging 2011;33:713–718.
18. Henkelman RM, Huang X, Xiang QS, et al. Quantitative interpretation of magnetization transfer. Magn Reson Med 1993;29:759–766.
19. Stanisz GJ, Webb S, Munro CA, et al. MR properties of excised neural tissue following experimentally induced inflammation. Magn Reson Med 2004;51:473–479.
20. Stanisz GJ, Kecojevic A, Bronskill MJ, Henkelman RM. Characterizing white matter with magnetization transfer and T(2). Magn Reson Med 1999;42:1128–1136.
21. Li T-Q, Yao B, van Gelderen P, et al. Characterization of T(2)* heterogeneity in human brain white matter. Magn Reson Med 2009;62:1652–1657.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Data S1. Supplementary methods.
Table S1. MND HIV patients clinical characteristics.
Table S2. Multivariate analysis of variance (MANOVA) for WM and GM T2* rt and MTR.

