Quantification of Acute Peripheral Muscle Edema in Rats using MRI

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Introduction

Vasodilatory leg edema is a common side effect of anti-hypertensive therapy using calcium channel blockers (CCB). Edema formation is currently determined after long-term clinical trials by measuring patient reported edema incidence, a fairly subjective parameter. Being able to assess peripheral edema in the pre-clinical setting would allow for earlier compound differentiation in the drug discovery process. In this study, we investigated the effects of reference compounds of CCB family nifedipine, mibefradil, and verapamil on the acute development of peripheral edema in rats using MRI.

Materials and Methods

Thirty-five Spontaneously Hypertensive Rats (SHR) (360 ± 65 g) were randomized into 10 groups (3 drugs at 3 doses each and control). Animal handling and surgery was performed in accordance to NIH guidelines and was approved by the local IACUC. Under general isoflurane anesthesia the rats' jugular vein and carotid artery were catheterized for drug delivery and blood pressure measurement. The animals were then placed prone on a heated bed with hind paws tightly secured to minimize motion artifact. Respiration and blood pressure were monitored and core temperature was maintained at 36 ± 1°C throughout the experiment. MRI was performed using a 7 Tesla Bruker horizontal magnet with a 12 cm gradient insert. A 72 mm birdcage volume coil was used for RF excitation and reception. A multi-slice multi-echo spin-echo sequence was employed to generate axial T2 maps. The imaging parameters were as follows: matrix size – 128×128, FOV = 45×55 mm, 16 slices, slice thickness 2 mm, 12 echoes at echo time spacing of 7 ms, repetition time 2500 ms, 2 averages. Each dataset acquisition took approximately 11 minutes. The animals' arterial blood pressure was allowed to stabilize for 30 minutes before the start of imaging. Two MRI datasets, 9 minutes apart, were acquired to establish the baseline condition. The rats were then dosed with nifedipine (2 (high), 0.2 (medium), 0.02 (low) mg/kg), mibefradil (20, 2, 0.2 mg/kg), verapamil (2, 0.2, 0.02 mg/kg), or vehicle (2 ml/kg) IV. Half of the compound dose was delivered as a bolus over 3 minutes, immediately followed by infusion of the other half of the dose over 60 minutes using a syringe infusion pump. Two more imaging datasets, 9 minutes apart, were acquired 30 minutes after the start of the infusion to assess the drug-induced T2 changes. Original MRI datasets were zero-padded to an in-plane matrix size of 256×256, 16 slices, assembled corresponding to the echo times and reconstructed to a final of 12 (one per echo) 3D images with a resolution 0.215 × 0.176 × 2 mm. Further image processing and analysis were performed with the AFNI software package (Cox RW, 1996). Percent increase in T2 during drug treatment was calculated voxel-by-voxel. To limit the number of false positives, all increases in T2 of less than 10% were set to zero. Representative color-coded images are shown in Figure 1. The sum of T2 percent increase values in all non-zero pixels over the carefully selected ROI (the integral T2 increase) was used as quantitative biomarker for the severity of edema. The blood pressure lowering (BPL) was measured as the difference of the mean arterial blood pressure before and after bolus administration and was used as a measure of drug efficacy. Statistical analysis was performed using ANOVA with the Student-Newman-Keuls (SNK) post-hoc multiple comparisons. Parameters with $P < 0.05$ were assumed to be statistically significant.

Results

Overall, the type of the treatment (drug) was found to have a statistically significant impact on blood pressure ($P = 0.018$) and edema ($P < 0.001$). The level of the treatment (dose) also had a significant overall effect on blood pressure ($P < 0.001$) and edema ($P < 0.001$). The interaction between drug and dose was also statistically significant (for blood pressure $P = 0.026$ and for edema $P = 0.005$). SNK test revealed that at a high dose level there were no differences between effects of all studied drugs on BPL, however, nifedipine caused a significantly higher T2 integral Increase. With a medium dose level of nifedipine the BPL was less than with the other drugs, whereas the T2 integral increase was significantly higher. At the low dose level neither BPL, nor the T2 integral increase was statistically different in all groups including control.

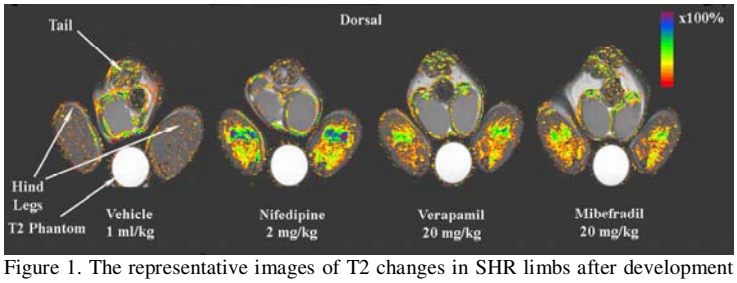

Figure 1. The representative images of T2 changes in SHR limbs after development of the acute edema. The statistically significant difference of T2 values before and after drug treatment in each pixel (color-coded image) is overlaid on the anatomical reference (grayscale) image.

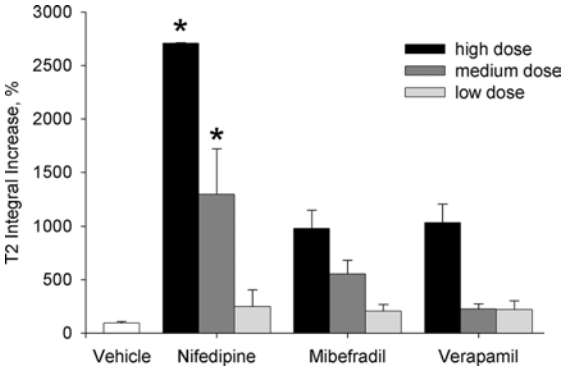

Figure 2. The effect of calcium channel blockers on peripheral muscle edema in SHR.
* - Statistically significant difference from other drugs within the dose level, $P < 0.05$

Discussion

The T2 proton relaxation depends strongly on the chemical and physical microenvironment and it is widely used to assess tissue water rearrangements consistent with edema in various organs both qualitatively and quantitatively (Josephson A et al, 2001; Ababneh Z et al, 2005). The novelty of our approach is the use of sequential T2 mapping of the rat hind limb muscle followed by threshold based testing of T2 changes pixel-by pixel in the same animal. The IV route for drug delivery and the acute nature of the edema formation in our study made this approach feasible. We believe that it is the ability to track T2 changes voxel-by-voxel that makes this approach very sensitive. To the best of our knowledge, there is no other validated quantitative method for the pre-clinical assessment of peripheral edema. Based on the results, it is concluded that T2 integral increase can be used to quantify peripheral edema with high precision and reproducibility.

References

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