Quantitative assessment of MRI T_2 response to kainic acid neurotoxicity in rats *in vivo*

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Purpose

The aim of this study was to elaborate on the development of non-invasive MRI biomarker of neurotoxicity, which we recently have proposed [1]. The first step of the development of such biomarker would be the detailed assessment of its quantification. Here, using the example of kainic acid-treated rats as an established model of neurotoxicity [2] we select among three different approaches of the quantification of the changes in T_2.

Methods

Animal use protocol was approved by NCTR IACUC. Male Sprague-Dawley rats (N = 37, 364 ± 40 g) were used. 21 rats received a single dose of kainic acid (KA, 10 mg/kg, ip) and 16 animals were treated with the vehicle (saline, 2 ml/kg, ip). MRI was performed using 7 tesla Bruker Biospec AV III equipped with 4-channel array rat brain RF coil. Animals were anesthetized with isoflurane and the body temperature was kept at 37.3 ± 0.6°C. KA or saline were delivered during MRI via ip catheter. For T_2 mapping a multi-echo spin echo sequence was used (MTX $192 \times 192 \times 24$, FOV = $3.84 \times 3.84 \times 2.4$ cm, echo spacing = 15 ms, 16 echoes, TR = 6 s, NA = 1). T_2 maps were skull stripped and co-registered to the template image using surface registration in Analyze 11.0. Then T_2 maps were smoothed using 2D running averages filter and down-sampled to half of the original in-plane resolution, keeping the number of slices intact. Three methods of the quantification of T_2 changes due to KA administration were employed. I. *Thresholding*: all T_2 maps were arbitrarily thresholded – voxels with $T_2 \leq 72$ ms were counted as normal tissue, and voxels with $T_2 > 72$ ms were counted as lesion; II. *Statistical mapping*: all T_2 maps after the treatment were tested against the averaged baseline voxel-by-voxel using t-test with Bonferroni correction; III. *Difference with their own baseline*: for each individual rat the baseline T_2 map was subtracted from the T_2 map after the treatment, counting only the voxels with CV values in the averaged baseline less than 10%. At the end the animals were deeply perfused transcardially with 4% paraformaldehyde for further histopathological analysis.

Results

All animals showed varying degree of response to KA as judged by both MRI and histology ranging from no response to severe changes (Fig. 1). Only responder animals were chosen for further analysis. The rat was considered the responder if histological assessment in any part of the brain showed some pathological changes. There were 9 responders, 7 non-responders, and 5 were not classified due to early sacrifice (2 hrs), which was too early for morphological evidence [Switzer]. Figure 2 shows the summary comparison chart of all three methods of quantification. Note that the thresholding method (I) was not able to detect differences between groups at any time point, while statistical mapping (II) detected such differences 24 and 48 hours after KA and the difference with the own baseline method (III) has shown statistically significant differences between KA and control groups at all time points, including very early (2 hrs).

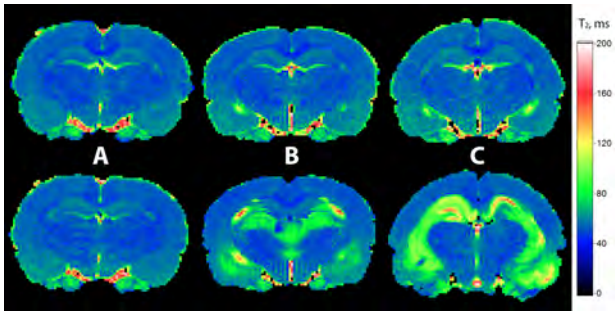

Figure 1. Examples of T_2 maps of the rat brains 48 hours after KA exposure. The severity of changes range from none (A), to medium (B) to severe (C), indicating different sensitivity of the animals to KA.

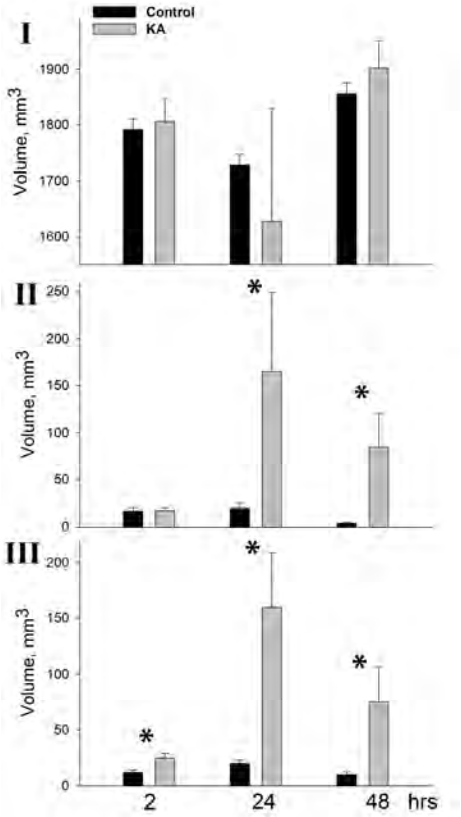

Figure 2. Results of three different methods of lesion volume quantification based on T_2 changes: I – thresholding; II – statistical mapping; III – difference with own baseline. * - significant difference between KA and control groups. Data are mean ± s.e.m.

Conclusion

T_2 mapping provides an easily quantifiable biomarker of neurotoxicity caused by KA and the quantification method employing the use of the same animal as its own control provides the most sensitive metrics. Future efforts will be applied towards the estimation of sensitivity and specificity of this biomarker against accepted 'golden standards'.

References

1. Hanig J., et al. Regul Toxicol Pharmacol. 2014, S0273-2300(14)00215-3.
2. Olney J. Can Dis Wkly Rep. 1990, 16 Suppl 1E: 47-57.
3. Switzer R.C., 3rd, et al. Toxicol Pathol. 2011, 39(1): 73-84.