# Map-ISODATA Demarcates Regional Response to Combination rt-PA and 7E3 F(ab')2 Treatment of Embolic Stroke in Rat

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**Introduction** ISODATA analysis using $T_1$, $T_2$, and ADCw maps (map-ISODATA) has proven to be a superior approach to ISODATA analysis using $T_1$- ($T_1$WI), $T_2$- ($T_2$WI), and diffusion- (DWI) weighted images (WI-ISODATA)[1]. In the present study, we investigate the ability of map-ISODATA to classify the different categories of ischemic damage in the lesion area and to evaluate combined thrombolytic (rt-PA) and antiplatelet (7E3 F(ab')$_2$) intervention after embolic stroke in rat.

**Materials and Methods** Male Wistar rats (300-350g) subjected to embolic stroke with (n=12) and without (n=10) rt-PA and 7E3 F(ab')$_2$ treatment (4 hours post-MCAO) were followed (at 2, 24 and 48h post-MCAO) with MRI using $T_1$, $T_2$, and $ADC_w$. Ischemic tissue damage was quantitatively analyzed on the immunostained and H&E stained slices. ISODATA was computed from $T_1$, $T_2$ and ADCw maps. The signatures characterized by the map-ISOADATA were compared with histological evaluation and were employed to demarcate the specific regions in the lesion.

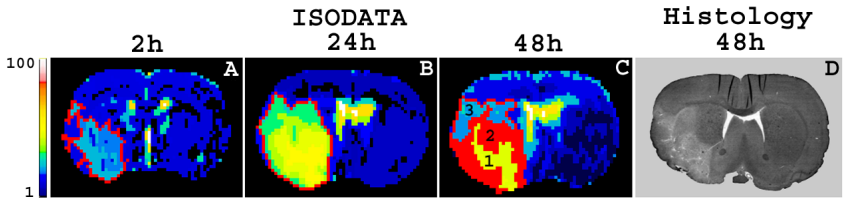


**Fig. 1** Map-ISODATA (**A**, **B** & **C**), specific regions (**C**; 1, 2 & 3) in the lesion and histology (**D**).

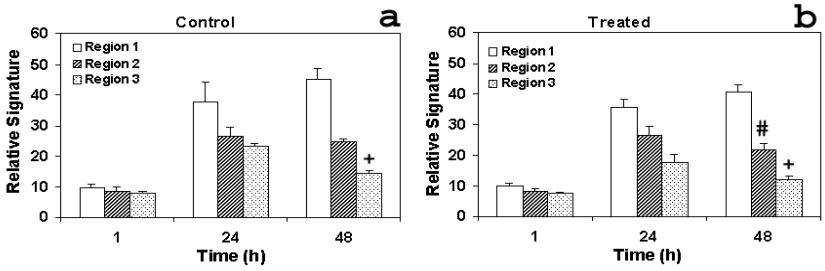


**Fig. 2** Signature evolution in three specific regions and comparison between control (**a**) and treated (**b**) groups. Significance of difference: + = $p < 0.05$, comparing 24h with 48h in "Region 3". # = $p < 0.05$, comparing 24h with 48h in "Region 2".

**Results** Our data indicate that the signature described by map-ISODATA is significantly correlated (R=-0.82) with the cell number of morphologically intact neurons in the lesion, suggesting that the signature value reflects and quantitatively grades the degree of tissue damage in the lesion area. Based on the segmentation and signature values provided by map-ISODATA at 48h after onset of embolic stroke, the lesion area was divided into three specific regions (Fig. 1C) for each animal, "Region 1", "Region 2" and "Region 3" assigned, respectively, to severely, moderately and least injured regions on the ISODATA. We used the relative signature (lesion signature normalized to the contralateral side) as an index to distinguish the injury levels of tissue in the lesion. Areas with relative signatures equal or larger than 30 identified "Region 1". Similarly, areas with relative signatures from 18 to 30, and equal or lower than 18 formed "Region 2" and "Region 3", respectively.

By using the partitions based upon the signature levels in map-ISODATA, $T_1$, $T_2$ and ADCw were sorted in an expected order. Higher signature values corresponded to higher $T_1$ and $T_2$ increments, and a greater change of ADCw throughout the time course studied, indicating that the region with higher signature level was more severely injured by ischemia[2-3].

The group comparison of temporal signature evolution within each of the three categories of ischemic damage is given in Fig. 2. The signatures in "Region 1" for both groups increase during the time course studied, while the signatures in "Region 3" for both groups decline ($p < 0.05$) at 48h after reaching the maximum values at 24h (Fig. 2a and 2b). The combination treatment results in a significantly reduced signature ($p < 0.05$) in "Region 2" at 48h compared with 24h post-MCAO (Fig. 2b). The changes of ADCw in "Region 1" also indicate that for both groups, this region is severely affected by the ischemia at an early stage of stroke, and tissue changes worsen with time, with or without treatment. Map-ISODATA can distinctly identify this specific region. We demonstrate that map-ISODATA is not only more accurate in identifying lesion areas (R=0.92 for map-ISODATA to correlate with the histological evaluation) than individual parameter maps (R=0.82, 0.86 and 0.51 for $T_1$, $T_2$ and ADCw maps, respectively), but is also more sensitive in detecting the evolution of tissue changes in both treated and non-treated conditions.

A significant overall group effect for lesion size ($p = 0.03$) and for signature mean value ($p = 0.047$) in the lesion was detected, with the treated group exhibiting a significantly smaller lesion size ($p < 0.05$) at 24 and 48h and a significantly lower signature mean value ($p < 0.05$) at 48h compared with the control group.

**Discussion** Map-ISODATA provides an accurate means to identify lesion area, to distinguish ischemic damage, to trace infarction evolution, and to detect the treatment response. Based upon map-ISODATA, ischemic lesion area can be divided into specific regions, each characterized by a distinct evolution of injury and treatment response. The central core of the ischemic lesion appears as a severely damaged region, with little hope of being affected by the treatment. Of the remaining moderate and least ischemia-affected regions, the former is a treatment-sensitive region, and the latter has self-mending potential. These data are consistent with observations that severe injury or an irreversible damage usually occurs in the central core of ischemic area[2-3]. In contrast, MR parameters in the least ischemia-damaged area can return near to the preischemic levels at a late stage of stroke[2-3]. Our data suggest that although the least ischemia-affected region has the highest chance to improve, the region immediately adjacent to this area and with denser ischemia can also respond to the combined treatment at a later stage of stroke. 7E3 F(ab')$_2$ extends the rt-PA treatment window to at least 4 hours after the onset of embolic stroke of rat.

**References**

1. Ding GL, Jiang Q, Zhang L, et al. Multiparametric ISODATA analysis of embolic stroke in rat. Journal of Neurological Sciences 2004; 223:135-143.
2. Knight RA, Dereski M., Helpern JA, et al. Magnetic resonance imaging assessment of evolving focal cerebral ischemia: Comparison with histopathology in rats. Stroke 1994; 25(6): 1252-1262.
3. Jiang Q, Zhang ZG, Chopp M, et al. Temporal evolution and spatial distribution of the diffusion constant of water in rat brain after transient middle cerebral artery occlusion. Journal of Neurological Sciences 1993; 120: 123-130.