Magnetic resonance imaging of malignant glioma using 5-aminolevulinic acid in an animal model

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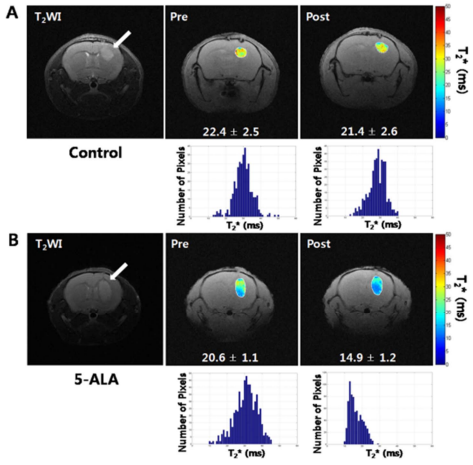
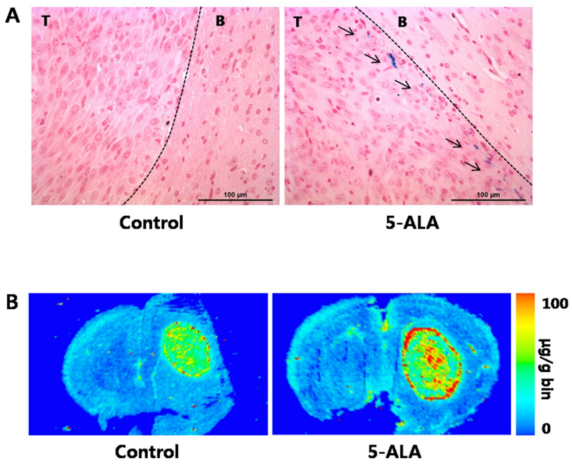
Purpose: We report the use of 5- aminolevulinic acid (ALA) for the noninvasive detection of malignant glioma in vivo using MRI. We hypothesized that 5-ALA would selectively accumulate in malignant glioma cells and be converted to heme, which could then be detected by MRI. To this end, we developed an orthotropic mouse brain tumor model using the human glioblastoma cell line U-87MG. In addition, we confirmed the accumulation of iron in malignant glioma cells using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS).
Materials and methods: For in vitro study, first, the human glioblastoma U-87 cells were incubated for 48 h with FAC of 100 μM for iron supplementation, or without FAC supplement. Second, the cells were incubated for 6 h with 5-ALA (500uM) or without 5-ALA, and then we measured intracellular PpIX. Third, the cells were further incubated for 1, 24, or 48 h. The harvested cells at the 1, 24, or 48 h after incubation were used for the measurement of intracellular concentration of iron, heme, PpIX, and ferrochelatase (FECH. A total number of 10 mice with orthotopic brain tumor were used. The mice received an oral administration of 5-ALA of 0.1mg/g(n=6; 5-ALA group) or normal saline (n=4; control group) 24 h before MRI (7 T MRI scanner, Bruker). We measured T2* of each tumor, and iron concentration of tumors was evaluated by LA-ICP-MS and histopathology.
Results: The intracellular PpIX concentration of the cells treated with 5-ALA was significantly higher than that of control cells (P <.001). The intracellular heme concentration of the cells with exposure to 5-ALA was higher than that of the cells without exposure only at 24 h (P<.001). The intracellular concentration of FECH was the highest at 24 h after exposure to 5-ALA, and decreased over time (48 h). In vivo T2* maps showed that the mean T2* value of U87 glioblastoma treated with 5-ALA was lower than that of control tumors (14.9±1.2 msec vs 21.4±2.6, P<.0001), which was well correlated with LA-ICP-MS and Prussian blue staining.
Conclusion: This study is the first to demonstrate that 5-ALA administration increases the intracellular iron concentration of glioblastomas by promoting the synthesis of heme, which is the metabolite of 5-ALA. As intracellular iron can be detected by MRI, we believe that 5-ALA-enhanced MRI will aid in the identification of high-grade foci in gliomas.



MRI detection of heme synthesized as a result of PpIX generated by 5-ALA treatment in U-87 glioblastomas. (A, B) The number of pixels (blue bars) was used to calculate the mean T_2* value, and these values were used for the detection of heme-induced changes in T_2*. The mean T_2* value of U-87 glioblastomas treated with 5-ALA was lower than that of control tumors, which received normal saline (P < 0.0001).



Determination of heme iron in brain tumors by histological analysis. (A) Multifocal iron deposits (arrow) at the border (dotted line) between the tumor and the brain in mice treated with 5-ALA were stained by Prussian blue. No staining was observed in the control mice treated with saline. (B) Intratumoral iron levels showed a significant difference in the mean concentration of iron between the control animals and the 5-ALA-treated animals, as measured by LA-ICP-MS (41.4 ± 0.9 μg/g vs. 60.1 ± 2.2 μg/g, P < 0.0001). T, tumor; B, brain.

References

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