Original Article

In Vivo 3T Magnetic Resonance Imaging (MRI) of Rat Brain Glioma-Bearing Tumor: A Comparison with Digital Caliper Measurement and Histology

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Abstract

Purpose: A major obstacle in brain tumor surgery is to precisely identify the boundaries of the tumor for maximal elimination without any residual tumoral tissues. Gliomas have the same color and texture as the normal brain tissues, which impose a major burden for neurosurgery. Recently, 3 Tesla Magnetic Resonance Imaging (MRI) scanners have demonstrated the potential for sub-millimeter anatomical imaging. Considering the growing importance of small animal imaging in pre-clinical research, the purpose of the present study was to evaluate the ability of contrast-enhanced MRI to determine glioma tumor volume in comparison with Caliper and Histology.

Materials and Methods: Thirteen rats were anesthetized, and fixed in stereotactic frame. C6 cells were injected into the right cortex of rats. Fourteen days later, rats were evaluated with 3 Tesla MRI equipped with a head receiver coil. Two hours before imaging, the Magnetic Nano-Particles (MNPs) were injected through the tail of the rats, before they were placed in a magnetic field (1.3 Tesla). Axial and coronal sections of the brain were obtained with a T2-weighted turbo-spin-echo protocol. Finally, rats were sacrificed and their brains were fixed in formalin for measuring the tumor volume with Caliper, and Hematoxylin and Eosin (H&E) staining.

Results: The results of calculating tumor volume by MRI, caliper and H&E were 72.6±7.3mm^3, 61±11.1mm^3 and 76.4±7.8mm^3, respectively. There was no significant difference in tumor volume between MRI and H&E ($P= 0.24$), while, there was a significant difference between MRI and caliper data ($P< 0.05$).

Conclusion: According to the results, it is not easy to discriminate the delicate border between normal tissue and Glioma tumor by the naked eye. However, H&E staining may help separate the boundary between normal tissue and tumor with a high precision at the cellular level. Comparing the results of 3 Tesla MRI with both the results from H&E and caliper indicated that there was not a significant difference between the findings of MRI an H&E staining, thus, MRI could be recognized as an acceptable method for non-invasive tumor volume measurement.

1. Introduction

Glioblastoma Multiforme (GBM) is the most malignant and invasive tumor of the brain. Despite the development of conventional treatments, the treatment is performed infrequently due to delayed and very poor diagnosis, and high recurrence rates [1]. Therefore, early diagnosis of glioma in the treatment can be decisive. Recent studies

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have focused on improving imaging techniques to achieve an ideal and non-invasive approach for determining the target volume [2]. Considering the growing importance of small animal imaging in pre-clinical research, recent efforts have been made to develop a variety of in-vivo diagnostic imaging methods [3]. Imaging techniques such as Positron Emission Tomography (PET), Single-Photon Emission Computed Tomography (SPECT) and high field MR scanners have been developed for functional and metabolic imaging in small animals [4, 5]. In addition, micro-CT systems can also be used for studies on morphology and diseases of small animals and rodents [3]. As de Crespigny *et al*. showed, the results of measuring the glioma tumor volume of the mouse using micro-CT imaging are close to the histology results [6]. However, in most studies, it has been demonstrated that MRI is more sensitive than CT imaging in detection of the lesions and the margin of glioma [7]. Recently, 3 Tesla MRI scanners with a dedicated animal coil have high potential for under-millimeter anatomical imaging, making them a powerful and cost-effective tool for imaging animal models, in order to simulate human malignancies, such as glioma brain tumors [8]. The automatic segmentation feature of MRI offers a potential for accurate determination of tumor volume and provides a reproducible standardized measurement protocol that can be used prior to brain tumor treatments [9]. Therefore, due to the importance of optimal imaging in clinical research, the aim of this study was to evaluate the ability of contrast-enhanced MRI in measuring the glioma tumor volume compared to that of histology staining as the gold standard method in the rat glioma model. In H&E staining, the differentiation of the tumor is based on the molecular, cellular, and structural differences between the glioma and the healthy tissue. Therefore, it is a reliably accurate method for determining tumor volume. We also compared it with Caliper measurement method as the ground truth. In this study, we used Superparamagnetic Iron Oxide Nanoparticles (SPIONs) nanoparticles to improve the contrast of MR images. SPIONs can be dynamically tracked during MR imaging, since they can accumulate and be targeted at the tumor site using an external magnetic field [10, 11]. SPIONs can enhance proton spin–spin (T2/T2*) relaxation time, thus MRI can detect nanoparticles as real-time and improve the differentiation of malignancy from healthy brain tissues [12].

2. Materials and Methods

The C6 rat glioma cells and Male Wistar rats (200-220 g) were obtained from the Pasteur Institute of Iran. All animals care was carried out according to the guidelines of the animal research committee of Iran University with Ethics in Animal Experiments (No. IR.IUMS.REC 1394. 9221339201).

2.1. Synthesis of Magnetite Nanoparticles

SPIONs was synthetized using the chemical co-precipitation method [13]. For polymerization, 30 mg of SPIONs was dispersed in dichloromethane (DCM) using an ultrasonic bath. The emulsion was emulsified in the polymer PLGA (50:50) and Span 60 in DCM by ultrasonication in an ice-bath. The resulted emulsion was diluted in glycerin and water (24 ml) under stirring. At last, MNPs were centrifuged, and then were collected by an external magnet.

2.2. Rat Brain Tumor Model

The C6 glioma Cells were cultured in Ham's F-12 medium, supplemented with 10% FBS. For creating tumor modeling, rats were anesthetized by the injection of ketamine (75 mg/kg) and xylazine (5 mg/kg). Then, 10 µl serum-free Ham's F-12 medium containing 1×106 C6 cells were injected into the right cortex of the brain. After 14 days' post-implantation, rats were assessed by MRI for analyzing tumor volume.

2.3. Glioma Tumor Volume Measuring with MRI, Calliper and Histology Methods

All MRI images were acquired on a 3 Tesla scanner (MAGNETOM Prisma, Siemens, Germany) equipped with a rat head receiver coil at the National Brain Mapping Laboratory of Iran (NBML). In order to increase the contrast of the images, the MNPs were injected two hours before imaging to the tail vein of rats. The rats placed between two blocks neodymium–iron–boron permanent magnet with a field strength of 1.3 Tesla (for two hours) for accumulation of MNPs at tumor region. Axial and coronal sections of the rat brains were obtained using a T2-weighted turbo-spin-echo protocol, imaging parameters: TR= 2300ms, TE= 107ms and slice thickness= 1.5mm. MRI data were processed with an ITK-SNAP (3.4.) software, the Region of Interest (ROI)

of the glioma in all slices was identified to measure the total tumor volume [1]. Then, rats were sacrificed and brains were fixed in formalin for measuring the tumor size using a digital Caliper. Then, samples were embedded in paraffin and cryosections with 1m thickness. Slices were stained with Hematoxylin and Eosin (H&E) and tumor volume was calculated.

2.4. Statistics

Statistical analyses were performed using GraphPad Prism 6. Results were presented as the means± SD for each group. Significance among groups was determined by ANOVA. Differences were considered significant for $P<0.05$.

3. Results

Figure. 1 a & c shows a rat glioma tumor in the right frontal and in Figure. 1 b and d histology of the glioma brain tumor tissue is illustrated against the healthy tissues. Glioma tumor volume at the right prefrontal site is calculated by a digital Caliper and H&E staining.

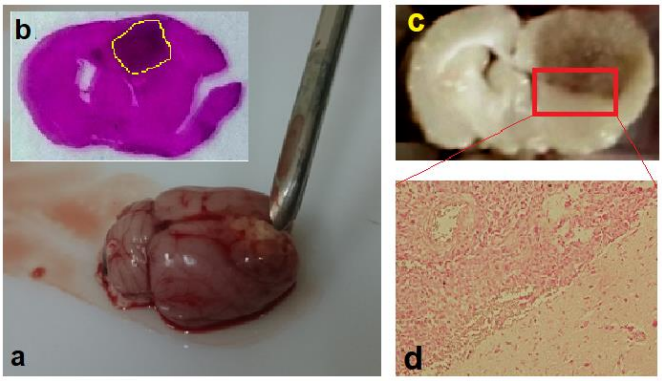

Figure 1. In-VIVO image of glioblastoma alograft. (a) Preparation of the brain for slicing (glioma tumor region in the rat brain was clearly identified), (b) The location of the GBM in the rat brain (the yellow dashed lines indicate glioma tumor area), (c) All brains were formalin fixed and , (d) Representative histological Hematoxylin and Eosin (H and E) staining of C6 glioma tumor

The MRI images of the glioma tumors are shown in Figures. 2a and b. The tumor was characterized by high intensity signals in T2-weighted images before injection of SPIONs/PLGA. After the injection of magnetic nanoparticles, the most significant change in contrast was observed in rat glioma sites (Figure. 2c). Tumor region intensity was distinct in post-injection of magnetic nanoparticle, indicating the enhanced negative contrast of SPIONs/PLGA.

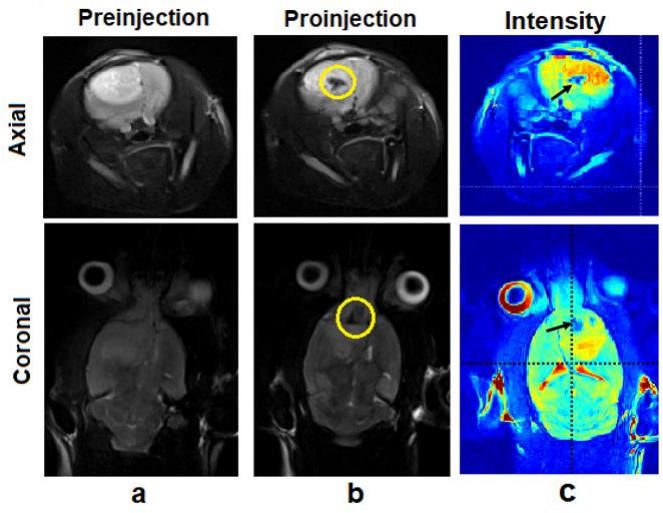

Figure 2. MR imaging of glioma rats enhanced by injection of SPIONs/PLGA. (a) MR coronal and axial images prior to and at 2-hour post-injection of SPIONs alone and (b) under external magnetic field. Yellow dash circle shows the location of the nanoparticle accumulation on glioma site, (c) Intensity changes of MR images for the tumor region, and the nanoparticle accumulation in tumor site illustrated with black arrow

Furthermore, a significant negative contrast enhancement noted by the applied magnetic field, shows that magnetic field can enhance the accumulation and retention of the SPIONs to the site of glioma. Based on MRI images, the tumor volume in each slice was calculated and the total volume was obtained.

The results of calculating tumor volume using a digital Calliper, 3 Tesla MRI and H&E staining at day 14 after tumor implantation were $61\pm11.1mm^3$, $72.6\pm7.3mm^3$, and $76.4\pm7.8mm^3$, respectively (Figure. 3). There was no significant difference in tumor volume between MRI and H&E ($P= 0.24$), while, there was a significant difference between MRI and Calliper data ($P< 0.05$), as well as between H&E and calliper ($P< 0.01$). Furthermore, there was a good correlation between the results of calculating tumor volume by MRI imaging and H&E staining ($r> 0.79$).

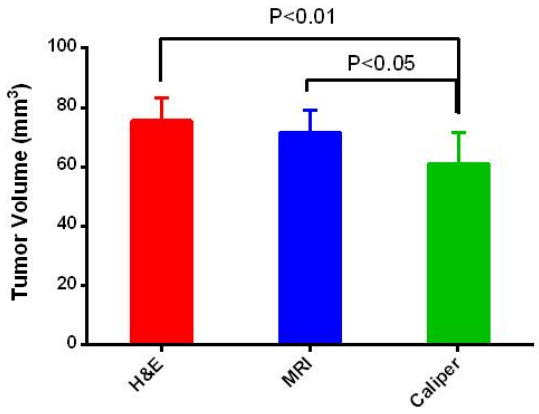

Figure 3. The calculated tumor volumes according to digital caliper, 3 Tesla MRI and H&E staining

4. Discussion

Despite the advances in imaging, the early detection of glioma, tracking glioma progression and analyzing the interaction of the glioma-brain are difficult due to the complex organization of the central nervous system [14]. In addition to detection of tumors, the main issue in treatment of GBM is the amount of tumor tissue removal, because in most cases, tumor recurrence occurs due to residual tumor tissue (even in small amounts) post-surgery. Various technologies may detect subtle differences between glioma and healthy brain tissue using ultrasound techniques, CT, MRI and biological evaluation of protein, DNA, vascular permeability and metabolic changes [15-17]. MRI navigation is an essential tool in brain surgery. Both MRI and CT imaging techniques are used for surgical navigation; however, MRI is preferred because it offers a better visualization of the tumor tissue and normal brain anatomy in most cases [17]. On the other hand, due to limited clinical studies, in vivo studies on real-time and non-invasive monitoring of brain tumors are major steps in neuro-oncological studies [18]. Ritman *et al*. proved that CT imaging in small animals can not reveal a glioma tumor due to lack of spatial resolution [19]. With the development of micro-CT systems, this contrast-enhanced equipment can get a complete brain scan within minutes, but it still needs to be fused with functional imaging systems [20]. Recently, magnetic resonance imaging systems have been modified to 7 T, and even MR systems have been specifically designed for imaging of small animals, especially for tracking glioma tumor with a 19-T magnetic field strength [15, 21]. Therefore, considering the importance of a precise and non-invasive method for determining tumor volume in the clinic, in this study, we used the three methods of digital Caliper, H&E staining and MRI imaging to determine the size of the rat glioma tumor. For this purpose, we used nanoparticles with PLGA polymer coating to increase the contrast of the MRI images. SPIONs have the ability to create a negative contrast in MRI images, as shown in Figure 2. These polymer nanoparticles have good stability in the in vivo condition, which is also studied by Mohammadi *et al.* and Esmaelbeygi *et al.* [22, 23]. The results of this study showed that there is a good correlation between the results of calculation of tumor volume with MRI imaging and H & E staining (Figure. 3), which is in agreement with the results of Engelhorn *et al* [3]. They also proved that there was no significant difference in tumor volume between micro-CT and MRI as well as between MRI and H&E staining. In addition, Figure 3 showed that there is a significant difference between the results of tumor volume measurements with H&E and MRI with digital Caliper measurement results ($P< 0.05$). The true border of the tumor was usually larger than what was depicted by digital Caliper, because measuring tumor volume by a digital Caliper is erroneous due to the presence of naked human eye [17]. This problem is even more serious in clinical practical applications. Since the texture and color of glioma tissue, especially low-grade tumors, are similar to that of healthy brain tissue, accurate tumor identification remains a challenge for neurosurgeons [24]. Therefore, in our opinion, histological evaluations remain more accurate than digital Caliper measurements although they have been served as ground truth in most applications. The diagnosis is based on tumor-specific cells and analysis with microscopy. Therefore, according to the results obtained in this study, MRI imaging can be a non-invasive method with a precision comparable to the Histological evaluation for the diagnosis of glioma tumors.

5. Conclusion

In recent years, clinical MRI systems or high field (up to 19 Tesla) MRI-scanners designed for small animal

imaging have been developed to glioma growth in vivo tracking. Therefore, comparing the results of enhanced 3 Tesla MR imaging equipped animal coil with both H&E staining (as gold standard) and caliper, showed that, MR imaging with a precision comparable to the H&E can be a reliable method for assessing the in vivo glioma tumor volume.

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