Aquaporin Positron Emission Tomography Differentiates Between Grade III and IV Human Astrocytoma

BACKGROUND: Aquaporin (AQP) water channels play a significant role in mesenchymal microvascular proliferation and infiltrative growth. AQPs are highly expressed in malignant astrocytomas, and a positive correlation is observed between their expression levels and histological tumor grade.

OBJECTIVE: To examine the utility of aquaporin positron emission tomography (PET) for differentiating between astrocytoma grade III and grade IV using the AQP radioligand (11C)TGN-020.

METHODS: Fifteen astrocytoma patients, grade III (n = 7) and grade IV (n = 8), and 10 healthy volunteers underwent (11C)TGN-020 aquaporin PET imaging. Surgical tissues of astrocytoma patients were examined for histopathological grading using the WHO classification standard and expression of AQP1 and AQP4 immunohistochemically.

RESULTS: Mean standardized uptake values of astrocytoma grade III and IV (0.51 ± 0.11 vs 1.50 ± 0.44, respectively) were higher than normal white matter (0.17 ± 0.02, P < .001) for both tumor grades. Importantly, mean standardized uptake values of astrocytoma grade IV were significantly higher than grade III (P < .01).

CONCLUSION: Our study demonstrated that (11C)TGN-020 aquaporin PET imaging differentiated between astrocytoma grades III and IV. We suggest its clinical application as a noninvasive diagnostic tool would lead to advancements in the management of these malignant brain tumors.

KEY WORDS Aquaporin1 (AQP1), Aquaporin4 (AQP4), Astrocytoma, [11C]TGN-020, Positron emission tomography (PET)

Aquaporins (AQPs) are ubiquitously expressed throughout the human body. AQP1 and AQP4 have an extremely regulated distribution in the central nervous system (CNS), where they play important roles in cerebrospinal fluid production and absorption and the regulation of blood-brain barrier water permeability. Recent studies have reported a relationship between astrocytoma malignancy and the abnormal expression of AQPs in the CNS, particularly AQP1 and AQP4.1,2 These water channel proteins are highly expressed in malignant astrocytomas,3,4 and a positive correlation is observed between their expression levels and histological tumor grade.5,6 Both AQPs have significant roles in mesenchymal microvascular proliferation and infiltrative growth.6-10 Indeed, malignant astrocytoma is characterized by microvascular proliferation and diffuse, infiltrative growth, tumor characteristics that are believed to promote tumor progression and portend a poor prognosis. Therefore, the evaluation of both the AQPs’ expression in malignant astrocytoma patients in vivo would lead to an early diagnosis of malignancy and an accurate evaluation of the extent of malignant tumor regions. However, real-time in vivo visualization of AQPs in brain tumor has not been reported previously.
TGN-020 is an AQP ligand that uniquely binds to AQP1 and AQP4. Its radioligand, $[^{11}C]TGN-020$, has previously successfully demonstrated the distribution of AQP in normal human brain using positron emission tomography (PET). In this study, we evaluated the AQP1 and AQP4 distribution in astrocytoma patients using $[^{11}C]TGN-020$ PET imaging and, furthermore, considered the difference in ligand uptake between grade III and IV astrocytoma.

**METHODS**

**Participants**

Malignant astrocytoma patients were recruited between November 2012 and October 2015 in accordance with the human research guidelines of the local Internal Review Board. All patients underwent gross total resection surgeries within a week of PET imaging, and none were treated using steroids. Histopathologic tumor diagnosis was completed based on the surgical specimens. Of the 23 patients recruited into this study, 5 were excluded for other tumor diagnoses; 4 oligodendroglioma and 1 central neurocytoma. In addition, 3 diffuse astrocytoma (WHO grade II) patients were excluded from the statistical analysis due to the limited sample size. Finally, the remaining 15 high-grade astrocytoma patients (age 29-81 yr, 48.3 ± 14.5), grade III (4 males and 3 females) and grade IV (5 males and 3 females), and 10 healthy volunteers (age 24-85 yr, 46.2 ± 19.5, 6 males and 4 females) participated in this study and were included in all statistical analyses. Written informed consent was obtained from each participant prior to the PET study. Informed consent included the description and intended use of $[^{11}C]TGN-020$, an unlabeled product. This trial was registered at the UMIN Clinical Trials Registry as UMIN000005626 (http://www.umin.ac.jp/ctr/index.htm).

$[^{11}C]TGN-020$ Synthesis

The radioligand, $[^{11}C]TGN-020$, was prepared using a TRACERlab FXc Versatile Automated Synthesizer (GE Healthcare, Schenectady, New York) as previously detailed. Quality control measurements on all solutions for human injection consisted of pH, chemical purity, radiochemical purity, and endotoxin tests. All $[^{11}C]TGN-020$ samples used in this study had chemical and radiochemical purities > 95%, with radiochemical yields between 400 and 600 MBq.

**PET Images**

The $[^{11}C]TGN-020$ PET/computed tomography (CT) scan was acquired using a combination PET/CT scanner (Discovery ST Elite, GE Healthcare). Low-dose CT scans were performed in helical mode with 120 kVp, 50 mA, helical thickness of 3.75 mm, and 15 cm field of view positioned in the region of the cerebrum. $[^{11}C]TGN-020$ (160-272 MBq, 2.2-4.2 MBq/kg body weight) was administered intravenously in 2 min by syringe pump (PHD2000, Harvard, Cambridge, Massachusetts). PET emission data were acquired over 30 min in 3-dimensional (3D) statistic mode, from 10 min after the administration of $[^{11}C]TGN-020$, with a 25.6 cm axial field of view. The emission scans were reconstructed with a 128×128×47 matrix (a voxel size of 2.0×2.0×3.27 mm) using a 3D ordered subset expectation maximization iterative reconstruction algorithm (2 iterations and 28 subsets) after attenuation correction using the CT data. All PET images were transferred to a workstation Xeleris 3.1 (GE Healthcare) for analysis. Tissue activity concentration was expressed as the standardized uptake value (SUV), g/ml, corrected for subject’s body weight and administrated dose of radioactivity.

**Statistical Analysis**

Three investigators, including a board-certified neuroradiologist and a board-certified neurosurgeon, drew the regions of interest (ROIs) using Volumetrix M1 on a workstation Xeleris 3.1, in reference to tumor regions in the magnetic resonance imaging (MRI) images. We set an SUV 0.3 or more as the tumor-positive regions in the software. Comparison of mean SUVs between parietal white matter in normal volunteers and grade III and grade IV astrocytoma in patients, respectively, was performed using the Mann–Whitney U-test (2-tailed). P values of .01 or lower were considered to be statistically significant. Analyses were performed using IBM SPSS Statistics 22.0 (IBM Corporation, Armonk, New York). P values were corrected for multiple comparisons by Bonferroni method.

**Histopathologic Tumor Grading and Immunohistochemistry**

Surgical specimens taken from patients were fixed in 20% formalin, phosphate buffered. Tumor grading was assessed by histopathological examination of surgical tissue according to the WHO classification standard for diffuse astrocytoma grade III and grade IV. Immunohistochemistry was performed on paraffin-embedded, 4 μm thick sections with primary antibodies against AQP1 (monoclonal; ab168387, Abcam, Cambridge, United Kingdom) and AQP4 (polyclonal; gift from Dr Kenji Sakimura15). Reactivity was visualized using the avidin-biotin-peroxidase complex method (Vector, California). Diaminobenzidine was used as the chromogen. Immunoreactivity (IR) of both AQP1 and AQP4 in tumorous region were assessed semiquantitatively and scored on a 3-point scale (immunoreactivity score: 1, weak; 2, moderate; 3, intense). The 3-point scale assessments were performed by 2 neuropathologists. The scores were analyzed using the Mann–Whitney U-test (2-tailed), and P values of .05 or lower were considered to be statistically significant.

**RESULTS**

T2-weighted MRI and $[^{11}C]TGN-020$ PET images are shown in Figure 1. Scale bars (right side) indicate corresponding SUV (g/ml). Astrocytoma grades III and IV showed more intense qualitative intratumoral $[^{11}C]TGN-020$ uptake compared to normal white matter area. Furthermore, $[^{11}C]TGN-020$ uptake in astrocytoma grade IV was more intense than in grade III.

Mean SUVs for normal volunteer white matter and astrocytoma grades III and IV are presented quantitatively in Figure 2. Mean SUVs of astrocytoma grades III and IV (0.51 ± 0.11 and 1.50 ± 0.44, respectively) were higher than that of normal white matter (0.17 ± 0.02, P < .001) for both tumor grades. Importantly, mean SUVs of astrocytoma grade IV were significantly higher than those of grade III (P < .01).

Immunohistochemical expression of AQP1 and AQP4 was detected in all surgical specimens taken from patients with WHO astrocytoma classification grade III (n = 7) and grade IV (n = 8). AQP1 IR was observed in the cytoplasm and proximal processes of the tumor cells, whereas AQP4 IR was more conspicuous in cell processes and endfeet attaching to blood vessels. Semi-quantified...
IR-scores of both AQP1 and AQP4, which were assessed on a 3-point scale (IR-score: 1, weak; 2, moderate; 3, intense), showed significant increases in grade IV compared to grade III (AQP1: 1.7 ± 0.49 vs 2.6 ± 0.53, AQP4: 1.9 ± 0.69 vs 2.9 ± 0.38, grade III vs grade IV, \( P < .05 \), respectively; Figure 3).

**DISCUSSION**

AQP1 is highly expressed along the vascular lumen in peripheral tissues. In the CNS, however, vascular AQP1 expression is completely suppressed. The normal distribution of AQP1 in the CNS is instead limited to choroid plexus epithelial cells. Moreover, there is virtually no expression of AQP1 in healthy astrocytes. By contrast, AQP4 is widely expressed throughout the CNS, primarily along astrocyte endfeet membranes contacting the basal lamina surrounding capillaries and dura, as well as, secondarily, along the processes of astrocyte endfeet.

In malignant astrocytoma grades III and IV, active suppression of vascular AQP1 is lost, leading to significant AQP1 expression in proliferating microvessels and clinically correlating to loss of blood-brain barrier integrity. Studies have shown that AQP1 expression in vessels enhance tumor cell migration. Indeed, a significant reduction in tumor growth and angiogenesis is found in AQP1-deficient mice. Increased AQP4 expression is also evident in astrocytoma cells, particularly when there is microvascular proliferation. Unlike normal AQP4 distribution on endfeet membranes contacting the basal lamina, astrocytoma AQP4 distribution is more uniform along endfeet membranes that are not in contact with the basal lamina. This altered AQP4 expression pattern appears to correlate with infiltration of tumor cells into surrounding healthy tissue. Alterations in AQP1 and AQP4 expression in malignant astrocytoma thus correlate with malignant properties, ie, tumor cell infiltration and angiogenesis. The latter is thought to be the result of epithelial-mesenchymal transition, histopathologically recognized as mesenchymal microvascular proliferation.

The current study demonstrated that astrocytoma grades III and IV had significantly higher uptake of the aquaporin PET ligand \([^{11}\text{C}]\text{TGN-020}\) relative to normal white matter, though the results are preliminary due to the small sample size. Importantly, uptake increase was proportional to tumor grade, clearly differentiating the 2 tumor grades. Immunohistochemical studies confirmed that astrocytoma grades III and IV were associated with a dramatic increase in AQP1 and AQP4 expression and distribution compared to normal tissue.

Clinically, the term malignant astrocytoma refers to highly aggressive tumor characterized by infiltration and mesenchymal microvasculature proliferation. Although most common malignant astrocytoma is glioblastoma multiforme (WHO grade IV), anaplastic astrocytoma (WHO grade III) may also be included. The results of this study demonstrated a clear
AQUAPORIN PET DISTINGUISHES ASTROCYTOMA MALIGNANCY

**CONCLUSION**

Our study demonstrated that \( ^{11} \text{C} \)TGN-020 aquaporin PET imaging differentiates between astrocytoma grades III and IV noninvasively. Its clinical application could help advance the management of these malignant brain tumors.

**Disclosures**

This study was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology (Japan) and University of Niigata. The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

**REFERENCES**

1. Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP. Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J Neurosci*. 1997;17(1):171-180.

2. Nielsen S, Smith BL, Christensen EI, Agre P. Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. *Proc Natl Acad Sci U S A*. 1993;90(15):7275-7279.

3. Saadoun S, Papadopoulos MC, Davies DC, Bell BA, Krishna S. Increased aquaporin 1 water channel expression in human brain tumors. *Br J Cancer*. 2002;87(6):621-623.

4. Saadoun S, Papadopoulos MC, Davies DC, Bell BA, Krishna S. Increased aquaporin-4 expression is increased in oedematous human brain tumours. *J Neurol Neurosurg Psychiatry*. 2002;72(3):262-265.

5. Warth A, Kröger S, Wolburg H. Redistribution of aquaporin-4 in human glioblastoma correlates with loss of agrin immunoreactivity from brain capillary basal laminae. *Acta Neuropathol*. 2004;107(4):311-318.

6. El Hindy N, Bankfalvi A, Herring A, et al. Correlation of aquaporin-1 water channel protein expression with tumor angiogenesis in human astrocytoma. *Anticancer Res*. 2013;33(2):609-613.

7. McCoy E, Sontheimer H. Expression and function of water channels (aquaporins) in migrating malignant astrocytes. *Glia*. 2007;55(10):1034-1043.

**FIGURE 3.** Immunohistochemistry of resected lesional tissue. A, Representative pictures of immunohistochemical staining of AQP1 (upper panel) and AQP4 (lower panel). Each of those was classified semiquantitatively and scored on a 3-point scale (IR-score). Scale bars: 20 μm. B, Comparison between grade III and grade IV IR-scores in AQP1 and AQP4. Mean ± SD *P < .05, Mann–Whitney U-test (2-tailed).
8. McCoy ES, Haas BR, Sontheimer H. Water permeability through aquaporin-4 is regulated by protein kinase C and becomes rate-limiting for glioma invasion. *Neuroscience*. 2010;168(4):971–981.

9. Ding T, Ma Y, Li W, et al. Role of aquaporin-4 in the regulation of migration and invasion of human glioma cells. *Int J Oncol*. 2011;38(6):1521–1531.

10. Saadoun S, Papadopoulos MC, Watanabe H, Yan D, Manley GT, Verkman AS. Involvement of aquaporin-4 in astroglial cell migration and glial scar formation. *J Cell Sci*. 2005;118(pt 24):5691–5698.

11. Huber VJ, Tsujiita M, Nakada T. Identification of aquaporin 4 inhibitors using in vitro and in vivo methods. *Bioorg Med Chem*. 2009;17(1):411–417.

12. Huber VJ, Tsujiita M, Nakada T. Aquaporins in drug discovery and pharmacotherapy. *Mol Aspects Med*. 2012;33(5-6):691–703.

13. Suzuki Y, Nakamura Y, Yamada K, Huber VJ, Tsujiita M, Nakada T. Aquaporin-4 positron emission tomography imaging of the human brain: first report. *J Neuroimag Eng*. 2013;23(2):210–233.

14. Nakamura Y, Suzuki Y, Tsujiita M, Huber VJ, Yamada K, Nakada T. Development of a novel ligand, [11C]TGN-020, for aquaporin 4 positron emission tomography imaging. *ACS Chem Neurosci*. 2011;2(10):568–571.

15. Tanaka K, Tani T, Tanaka M, et al. Anti-aquaporin 4 antibody in selected Japanese multiple sclerosis patients with long spinal cord lesions. *Mult Scler*. 2007;13(7):850–855.

16. Saadoun S, Papadopoulos MC, Hara-Chikuma M, Verkman AS. Impairment of angiogenesis and cell migration by targeted aquaporin-1 gene disruption. *Nature*. 2005;434(7034):786–792.

17. Noell S, Wohlburg-Buchholz K, Mack AF, et al. Dynamics of expression patterns of AQP4, dystrocygan, agrin and matrix metalloproteinases in human glioblastoma. *Cell Tissue Res*. 2012;347(2):429–441.

18. Brat DJ. Astrocytic tumors. In: Love S, Budka H, Ironside JW, Perry A, eds. *Greenfield’s Neuropathology*, 9th ed. Boca Raton: CRC Press; 2015:1638–1672.

19. Milson CC, Yu JI, Mackman N, et al. Tissue factor regulation by epidermal growth factor receptor and epithelial-to-mesenchymal transitions: effect on tumor initiation and angiogenesis. *Cancer Res*. 2008;68(24):10068–10076.

20. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352(10):987–996.

21. Yool AJ, Brown EA, Flynn GA. Roles for novel pharmacological blockers of aquaporins in the treatment of brain oedema and cancer. *Clin Exp Pharmacol Physiol*. 2010;37(4):403–409.

**Acknowledgment**

The authors are very grateful to Mr Yuta Yagi for his help in PET ligand synthesis and PET image acquisition.

**COMMENTS**

In this article, the authors report preliminary findings regarding the ability of a novel molecular imaging technique, [11C] TGN-020 PET, to distinguish between grade III and grade IV malignant astrocytomas. TGN-020 is an aquaporin ligand that binds uniquely to AQP1 and AQP4, aquaporin proteins, which have recently been shown to be expressed in high levels in malignant astrocytomas. In a small cohort of 15 patients with high-grade astrocytomas (grades III and IV), the authors found that the tumors demonstrated a significantly higher mean standard uptake value (SUV) compared to normal white matter on [11C] TGN-020 PET and also, perhaps more importantly, that grade IV astrocytomas showed a significantly higher mean SUV than grade III astrocytomas. In addition, the authors were able to demonstrate significant differences in the immunohistochemical expression of both AQP1 and AQP4 between grade III and grade IV gliomas.

These findings suggest that [11C] TGN-020 PET could be a valuable diagnostic and prognostic tool for patients with primary brain tumors. Unfortunately, due to the constraints of the study, which was limited to a very small sample of grade III and IV gliomas, conclusions based on these results cannot be generalized beyond patients with high-grade tumors. In addition, although [11C] TGN-020 PET appears in this study to have been excellent at discriminating grade III and grade IV gliomas, it is reasonable to wonder how the technique stacks up for noninvasive tumor grading against other promising and widely available MR-based techniques, such as diffusion kurtosis imaging, dynamic contrast enhanced permeability imaging, and susceptibility weighted imaging (just to name a few). Ultimately, it remains to be seen whether and how [11C] TGN-020 PET will be used in clinical practice in the future.

It is also unfortunate that the authors did not incorporate genetic data on the tumors in this study in their analysis, particularly in light of what we now know about how IDH mutation status predicts clinical behavior in patients with glioblastoma. Nevertheless, [11C] TGN-020 PET appears to be an exciting new tool in neuro-oncologic imaging, and the findings in this study open the door to a number of avenues for future investigation, including studies on the potential value of aquaporin imaging for furthering our understanding glioma biology and pathogenesis, noninvasive tumor grading, prognosis determination, clinical management, and development of new therapeutic targets.

**Benjamin Huang**
Chapel Hill, North Carolina

---

1. Isokpehi RD, Wollenberg Valero KC, Graham BE, et al. Secondary data analytics of aquaporin expression levels in glioblastoma stem-like cells. *Cancer Inform*. 2015;14:95–103.

2. Johansson PA, Drziegielewska KM, Ek CJ et al. Aquaporin-1 in the choroid plexuses of developing mammalian brain. *Cell Tissue Res*. 2005;322(3):353–364.

3. Jaconetta C, Rudloff E, Kirby R. The role of aquaporin 4 in the brain. *Vitam Cell Pathol*. 2012;41(1):32–44.

4. Verkman AS, Anderson MO, Papadopoulos MC. Aquaporins: important but elusive drug targets. *Nat Rev Drug Discov*. 2014;13(4):259–277.