CASE REPORT

Evaluation of the Antiangiogenic Effects of Octreotide on Growth Hormone-producing Pituitary Adenoma using Arterial Spin-labeling Perfusion Imaging

Naoto SAKAI¹, Shuhei YAMASHITA², Yasuo TAKEHARA^{3*}, Harumi SAKAHARA²,
Satoshi BABA⁴, Yutaka OKI⁵, Goro TAKAHASHI⁶, Shinichiro KOIZUMI⁷,
Tetsuro SAMESHIMA¹, and Hiroki NAMBA¹

¹*Department of Neurosurgery, Hamamatsu University School of Medicine*

²*Department of Radiology, Hamamatsu University School of Medicine*

³*Department of Radiology, Hamamatsu University Hospital*

⁴*Department of Diagnostic Pathology, Hamamatsu University School of Medicine*

⁵*Second Department of Internal Medicine, Division of Endocrinology and Metabolism,
Hamamatsu University School of Medicine*

⁶*Department of Otorhinolaryngology, Division of Head and Neck Surgery, Hamamatsu University School of Medicine
1–20–1 Handayama, Higashi-ku, Hamamatsu, Shizuoka 431–3192, Japan*

⁷*Department of Neurosurgery, Seirei Mikatahara General Hospital*

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Decreased absolute tumor blood flow (TBF) measured by arterial spin labeling perfusion imaging (ASL-PI) on 3-tesla magnetic resonance imaging demonstrated the reduced size and growth hormone (GH) secretion of a large GH-producing pituitary adenoma in a 32-year-old man in response to octreotide therapy. The study shows the usefulness of ASL-PI in providing a biomarker of the antiangiogenic effect of octreotide.

Keywords: *angiogenesis, magnetic resonance, arterial spin-labeling (ASL), octreotide, pituitary adenoma*

Case Report

A 32-year-old man with a one-year history of visual disturbance was referred to our neurosurgical department for the investigation and treatment of a large and lobular pituitary adenoma that compressed the optic chiasm and invaded to the left cavernous sinus. Ophthalmologic examination showed his corrected visual acuity as 0.7 on the right side and 0.8 on the left with bitemporal hemianopia. He had typical clinical features of acromegaly that included enlarged doughy hands and feet, frontal bossing, coarsening of facial features with increased interdental spacing, and thickened lips. His basal level of plasma growth hormone (GH) was 56.6 ng/mL and of insulin-like growth factor (IGF-) 1, 586 ng/mL. An endocrinologist (Y.O.) evaluated the pa-

tient and diagnosed acromegaly. A test of octreotide suppression (measurement of GH for 24 hours following 100 µg subcutaneous administration of octreotide) showed good suppression of GH (from 79.0 ng/mL to 2.6 ng/mL at 12 hours). Because of the tumor's invasiveness and good response to octreotide, we administered 20 mg of long acting octreotide acetate for injectable suspension (Sandostatin® LAR, Novartis Pharmaceuticals Co., East Hanover, NJ, USA) before surgery.

The patient underwent conventional magnetic resonance (MR) imaging that included coronal and sagittal fat-suppressed T₁-weighted imaging (T₁WI), fat-suppressed T₂-weighted imaging (T₂WI), contrast-enhanced fat-suppressed T₁WI, 3-dimensional (3D) time-of-flight MR angiography (MRA), and pseudo-continuous arterial spin labeling perfusion imaging (ASL-PI) on a 3-tesla scanner (Discovery 750 3.0T: GE HC, Waukesha, WI, USA) 14 days before administration of octreotide and 21 days af-

*Corresponding author, Phone: +81-53-435-2242, Fax: +81-53-435-2241, E-mail: takehara@hama-med.ac.jp

Fig. 1. Growth hormone (GH)-producing pituitary adenoma in a 32-year-old man. (A, B) Sagittal and coronal post-contrast fat-suppressed T₁-weighted images (T₁WI) (repetition time [TR]/echo time [TE]/number of excitations [NEX], 600 ms/9.2 ms/3) show a large and invasive pituitary adenoma that extends superiorly to the optic chiasm and laterally into the left cavernous sinus. (C) Arterial spin-labeling perfusion imaging (ASL-Pi) (TR/TE/NEX, 4594 ms/10.6 ms/3; post-label delay, 1525 ms; slice thickness, 3.8 mm) using a 3-tesla scanner shows relatively high signal intensity at the pituitary tumor (arrow) (mean absolute tumor blood flow [mTBF], 115 mL/min/100 g; normalized tumor blood flow [nTBF], 2.2 mL/min/100 g). $nTBF = mTBF / (\text{right cerebellar blood flow [CBF]} + \text{left cerebellar CBF}) / 2$. (D, E) Sagittal and coronal post-contrast fat-suppressed T₁WI 14 days after octreotide administration shows remarkable tumor shrinkage. (F) ASL-Pi after octreotide administration shows remarkable reduction in tumor blood flow (arrow) (mTBF, 32 mL/min/100 g; nTBF, 0.7 mL/min/100 g).

ter its administration. The imaging parameters of ASL-Pi were: repetition time (TR)/echo time (TE)/number of excitations (NEX), 4594 ms/10.6 ms/3; receive bandwidth (RBW), 62.5 kHz; post-label delay, 1525 ms; slice thickness, 3.8 mm; number of slices, 32; field of view (FOV), 24 cm; and matrix, 512. The acquisition time for ASL-Pi was 3 min. We obtained his written informed consent prior to each MR study. To estimate tumor size, we averaged its 3 maximal dimensions (anterior-posterior, right-left, and superior-inferior) as determined by contrast-enhanced fat-suppressed T₁WI.¹ We also referred to those T₁WI findings to calculate the mean absolute tumor blood flow (mTBF, mL/min/100 g) in a region of interest (ROI) at the maximal axial cross-section of the pituitary adenoma on the ASL-Pi map. To evaluate normalized tumor blood flow (nTBF), we calculated the perfusion ratios of tumor to healthy tissue by dividing the mean value

of the tumor ROI by the mean value of ROIs in the bilateral cerebellar hemispheres (approximately 2.5 cm² each). MR imaging after the administration of octreotide demonstrated a remarkable decrease of tumor size (mean maximal diameter, from 4.6 to 3.6 cm) coupled with suppression of serum GH (from 56.6 to 1.53 ng/mL). The response of the pituitary adenoma to the octreotide was successfully reflected by the decreased mTBF (from 115 to 32 mL/min/100 g) and nTBF (from 2.2 to 0.7) (Fig. 1). We used a combined supra- and infra-sellar approach to remove the tumor. At surgery, the tumor was hypovascular and relatively firm. After surgery, the patient recovered from his visual disturbance, and his plasma levels were 0.87 ng/mL for GH and 158 ng/mL for IGH-1. An experienced pathologist (S.B.) examined hematoxylin and eosin-stained sections and immunohistochemical stains against adrenocorticotrophic hormone, prolactin, GH, luteinizing hor-

mone, follicle-stimulating hormone, and thyroid-stimulating hormone and diagnosed GH-producing pituitary adenoma. We immunostained the tissue section with somatostatin receptor (SSTR) 2, the most relevant receptor for octreotide binding, using the monoclonal mouse antihuman SSTR2 antibody (clone # 402038, R&D system), and observed it diffusely positive.

Discussion

Angiogenesis, the process by which new blood vessels are formed from pre-existing endothelial cells, plays a crucial role in tumor growth in promoting oxygenation, nutrient perfusion, and the removal of metabolic waste.² Recently, it was proposed that endothelial cells establish a vascular niche that promotes tumor growth and tissue repair through an angiocrine mechanism by promoting stem and progenitor cell-active trophogens.³ Vascularization in the pituitary gland, unlike other tissue, is lower in adenomas than the normal gland.^{4,5} Nevertheless, a relationship between angiogenesis and tumor size, invasiveness, and aggressiveness has been shown in some types of pituitary tumor.^{4–6} Inhibition of angiogenesis may become a target for therapy of these types of pituitary tumor.

Somatostatin is a peptide widely distributed throughout the central nervous system and peripheral tissues. The efficacy of somatostatin analogues in inhibiting tumor proliferation and hormone secretion depends on the tumor's expression of specific SSTR subtypes.⁷ Available data suggest they might inhibit angiogenesis directly through SSTRs on endothelial cells and indirectly through the inhibition of growth factors, such as vascular endothelial growth factor (VEGF).^{7–9} Vidal and colleagues evaluated microvascular density, a histopathological biomarker of angiogenesis, in various types of pituitary adenoma and showed a tendency for lower microvascular density of GH-producing adenomas treated with octreotide than those untreated; the octreotide is a somatostatin analogue that acts through selective binding to SSTR2 and reduces the serum concentration of GH and IGF-1 in most patients with acromegaly.¹⁰ Ideally, measurement of the biomarker of angiogenesis should be relatively easy by imaging. Although the utility of molecular MR imaging of VEGF (alphaVbeta3-targeted MR imaging) and VEGF receptor 2 has been reported for evaluating tumor angiogenesis in rodents, these methods have not been used in clinical settings.^{11,12}

ASL-PI involves the magnetic labeling of the spin population in arterial water by inversion and

its use as an endogenous diffusible tracer; this is a promising MR imaging-based method that leads to quantification of cerebral blood flow (CBF).^{13–16} A recent report of the technique's application to pituitary adenomas showed a correlation between tumor blood flow and microvascular density using immunohistochemistry for CD-31 antigen, a reliable marker of endothelial cells.¹ Treatment of our patient with octreotide LAR 2 weeks before surgery resulted in the remarkable reduction of tumor volume and blood flow coupled with decreased serum GH. We believe this is the first report that reveals the efficacy of ASL-PI in evaluating the antiangiogenic effect of octreotide on GH-producing pituitary adenomas.

In this study, we did not measure arterial blood transit time, which might be changed before and after the administration of octreotide. Its measurement would require the protocol to have varying post-labeling delays, which would prohibitively increase scan duration.¹⁷ Although further investigation is necessary to measure precise tumor blood flow using ASL-PI, such a labeling protocol might not be necessary to assess tumor angiogenesis in clinical settings.

In conclusion, ASL-PI has the potential to evaluate the effect of such antiangiogenic drugs as octreotide on pituitary adenomas. Although our findings require confirmation in larger prospective studies, we believe ASL-PI may greatly aid the management of patients with pituitary adenomas treated with antiangiogenic drugs.

References

1. Jahangiri A, Lamborn KR, Blevins L, Kunwar S, Aghi MK. Factors associated with delay to pituitary adenoma diagnosis in patients with visual loss. *J Neurosurg* 2012; 116:283–289.
2. Folkman J. Angiogenesis. *Annu Rev Med* 2006; 57: 1–18.
3. Butler JM, Kobayashi H, Rafii S. Instructive role of the vascular niche in promoting tumour growth and tissue repair by angiocrine factors. *Nat Rev Cancer* 2010; 10:138–146.
4. Turner HE, Nagy Z, Gatter KC, et al. Angiogenesis in pituitary adenomas and the normal pituitary gland. *J Clin Endocrinol Metab* 2000; 85:1159–1162.
5. Turner HE, Harris AL, Melmed S, Wass JS. Angiogenesis in endocrine tumors. *Endocr Rev* 2003; 24: 600–632.
6. Yamada S, Takada K. Angiogenesis in pituitary adenomas. *Microsc Res Tech* 2003; 60:236–243.
7. Garcia de la Torre N, Wass JA, Turner HE. Antiangiogenic effects of somatostatin analogues. *Clin Endocrinol (Oxf)* 2002; 57:425–441.

8. Kurosaki M, Saegert W, Abe T, Lüdecke DK. Expression of vascular endothelial growth factor in growth hormone-secreting pituitary adenomas: special reference to the octreotide treatment. *Neurol Res* 2008; 30:518–522.
 9. Zatelli MC, Piccin D, Vignali C, et al. Pasireotide, a multiple somatostatin receptor subtypes ligand, reduces cell viability in non-functioning pituitary adenomas by inhibiting vascular endothelial growth factor secretion. *Endocr Relat Cancer* 2007; 14:91–102.
 10. Vidal S, Kovacs K, Horvath E, et al. Microvessel density in pituitary adenomas and carcinomas. *Virchows Arch* 2001; 438:595–602.
 11. Sipkins DA, Cheresch DA, Kazemi MR, Nevin LM, Bednarski MD, Li KC. Detection of tumor angiogenesis *in vivo* by alphaVbeta3-targeted magnetic resonance imaging. *Nat Med* 1998; 4:623–626.
 12. He T, Smith N, Saunders D, et al. Molecular MRI differentiation of VEGF receptor-2 levels in C6 and RG2 glioma models. *Am J Nucl Med Molec Imaging* 2013; 3:300–311.
 13. Detre JA, Leigh JS, Williams DS, Koretsky AP. Perfusion imaging. *Magn Reson Med* 1992; 23:37–45.
 14. Detre JA, Zhang W, Roberts DA, et al. Tissue specific perfusion imaging using arterial spin labeling. *NMR Biomed* 1994; 7:75–82.
 15. Detre JA, Alsop DC. Perfusion magnetic resonance imaging with continuous arterial spin labeling: methods and clinical applications in the central nervous system. *Eur J Radiol* 1999; 30:115–124.
 16. Koretsky AP. Early development of arterial spin labeling to measure regional brain blood flow by MRI. *Neuroimage* 2012; 62:602–607.
 17. Xu G, Rowley HA, Wu G, et al. Reliability and precision of pseudo-continuous arterial spin labeling perfusion MRI on 3.0 T and comparison with 15O-water PET in elderly subjects at risk for Alzheimer's disease. *NMR Biomed* 2010; 23:286–293.
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