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Correlation of VEGF with Contrast Enhancement on Dual-Phase Dynamic Helical CT in Liver Tumors: Preliminary Study

The purpose of this preliminary study is to elucidate that vascular endothelial growth factor (VEGF) influences contrast enhancement of hepatic tumors on computed tomography (CT). Fourteen patients with hepatic tumors (11 hepatocellular carcinomas; 3 metastatic cancers) underwent a dual-phase dynamic helical CT or computed tomographic hepatic arteriography. The attenuation of each mass was determined as hyperattenuation, isoattenuation or hypoattenuation with respect to the adjacent nontumorous parenchyma. Gun-needle biopsy was done for each tumor, and paraffin sections were immunostained with anti- VEGF antibody by the avidin-biotin-peroxidase complex method. The pathologic grade was made by intensity (1+, 2+, 3+) and area (±, 1+, 2+). The tumor ranged 2.0-14.0 cm in size (mean, 5.8 cm). In arterial phase, the intensity was not correlated with the degree of enhancement (p=0.086). However, the correlation between the attenuation value of hepatic arterial phase and the area of positive tumor cells was statistically significant (p=0.002). VEGF may be the factor that enhances the hepatic mass with water-soluble iodinated contrast agent in CT.

Key Words: *Vascular Endothelial Growth Factor (VEGF); Computed Tomography, Contrast Enhancement; Liver; Liver Neoplasms; Liver Neoplasms, Blood Supply; Computed Tomography, Helical*

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INTRODUCTION

While the diagnostic benefits of water-soluble contrast agents are firmly established in computed tomography (CT), the pathophysiologic basis of the contrast enhancement and its histopathologic correlation remains vague. Several attempts have been made on contrast enhancement with respect to vascular density, but discrepant results were noted indicating that factors other than vascular density influenced the contrast enhancement (1, 2).

After intravenous administration, the contrast agents are rapidly distributed in the vascular and the interstitial space. When contrast agents are injected into an artery, the molecules leak from the capillary lumen by diffusion (3, 4). Therefore the degree of contrast enhancement of a lesion is dependent on the permeability of the microvasculature as well as the vessel density of the site. Recently it was reported that the differences in contrast enhancement patterns of tumors detectable by MRI are mainly due to vascular permeability, which leads to more characteristic differences than vessel density (5). Strugar et al. (6) reported a high correlation between the presence of vascular endothelial growth factor (VEGF) and the occurrence of peritumoral brain edema in cerebral metastases. All these properties suggest that VEGF is the histopathologic factor influencing contrast enhancement by angiogenesis and increased vascular permeability.

It is proposed that differences of enhancement patterns in radiologic studies are caused by different VEGF expression. If this hypothesis is true, VEGF expression should be highly correlated with enhancement patterns. The purpose of this preliminary study is to elucidate that VEGF influences contrast enhancement of hepatic tumor in CT.

MATERIALS AND METHODS

From May 1997 until April 1998, a prospective study was done with 21 biopsy specimens of solid liver masses from 21 patients which were detected and enhanced homogeneously on dual-phase dynamic helical CT or com-

puted tomographic hepatic arteriography (CTHA). The tumor showing enhancement mixed with hyperattenuation and hypoattenuation is excluded from the first. Fourteen specimens (from 14 patients; 12 males, 2 females; age range 35-67 yr; mean age, 52 yr) among them, which were properly biopsied and of which the diagnosis were made on hematoxylin-eosin (HE) staining, were included in this study. These specimens were subjected to immunohistochemical staining against VEGF. Histologic analysis revealed 11 hepatocellular carcinomas (HCCs) and 3 metastatic cancers (Table 1). Disposable automated biopsy gun, 18G Tru-Cut type (Solco Intermed, Seoul, Korea), was used for biopsy. Biopsy site was selected according to dual-phase dynamic helical CT (13 cases) or CTHA (1 case) findings, and was targeted with ultrasonographic guidance, so we could correlate the biopsy specimen with a mass on dual-phase dynamic helical CT or CTHA image. The biopsy was done, 2 or 3 times per a tumor, on 4 to 60 days (mean, 19 days) after dual-phase dynamic helical CT or CTHA. All scans were obtained by using a CT Picker 2000 (Picker International, OH, U.S.A.). Contrast material-enhanced dynamic helical CT of the entire liver was performed by using 8-mm collimation with 1:1 pitch in a craniocaudal direction. The scanning sequences were initiated 25 sec and 180 sec after the start of the intravenous injection of 100 mL of nonionic contrast material (Ultravist 300, Schering AG, Germany) at a rate of 3 mL/sec. The attenuation of each mass was determined as hyperattenuation, isoattenuation or hypoattenuation relative to the adjacent nontumorous parenchyma at window level 40 Hounsefield unit (HU) and width 300 HU.

To study the VEGF expression, immunohistochemical staining was performed using the avidin-biotin-peroxidase complex method. Briefly, after deparaffinized and rehydrated through graded alcohols, endogenous peroxidase activity was blocked with 3 % hydrogen peroxide for 10 min. The sections were incubated with an anti-VEGF monoclonal antibody (NeoMarkers, Union, CA, U.S.A.) at a dilution of 1:50 for 60 min. Subsequently, the slides were incubated with biotinylated secondary antibody for 30 min, followed by avidin-biotin-conjugated peroxidase complex for 30 min. Diaminobenzidine was used as a chromogen. Negative controls were established by replacing the primary antibody with normal rabbit serum.

Intensity of positive tumor cells was graded as weak intensity with pale brown color (1+), moderate intensity (2+), and strong intensity with dark brown color (3+). And scoring was performed according to the percentage (area) of positive tumor cells as scanty area (±, 10%), moderate area (1+, 10% to 50%), and diffuse area (2+, ≥50%). Two observers evaluated staining results independently, and differences in interpretation were resolved by discussion. For statistical analysis, Mantel-Haenszel chi square test was used.

RESULTS

The tumor ranged 2.0-14.0 cm in size (mean, 5.8 cm).

Table 1. Summary of clinical characteristics, CT contrast enhancement and VEGF staining of 14 patients

Patient No.	Sex	Age (yr)	Pathology	Interval (D)	Size (cm)	Dual-phase Dynamic Helical CT*		VEGF staining	
						Arterial Phase	Equilibrium Phase	Intensity†	Area‡
1	F	62	HCC	18	5.5	H	I	+++	++
2	M	41	HCC	6	3.5	H	L	+++/++	++
3	M	51	HCC	40	3	H	I	+++	++
4	M	67	HCC	23	2	H	L	+++	++
5	M	53	HCC	26	4	H	L	+++	++
6	M	51	HCC	4	6.5	H	I	++	++
7	M	41	HCC	17	8	I	L	+++	+
8	M	57	HCC	23	14	I	L	+++/++	++
9	M	55	HCC	5	7	L	L	+++	+
10	M	50	HCC	60	7	L	L	+	±
11	M	35	HCC	15	2	L	L	+++	±
12	M	53	Colon ca	5	6	L	L	+	±
13	F	50	Colon ca	23	3.5	L§		++/+++	+
14	M	66	Stomach ca	4	9	L	L	+/++	+

*H, hyperattenuation; I, isoattenuation; L, hypoattenuation

†Relative staining intensity was scored subjectively: 1+, weak intensity with pale brown color; 2+, moderate intensity; 3+, strong intensity with dark brown color. p=0.086 vs. arterial phase; p=0.538 vs. equilibrium phase

‡Relative staining area of positive tumor cells was scored subjectively: ±, <10%; 1+, 10% to 50%; 2+, ≥50%. p=0.002 vs. arterial phase; p=0.091 vs. equilibrium phase

§Computed tomographic hepatic arteriography

Hyperattenuation was seen in 6 cases, isoattenuation in 2 cases, hypoattenuation in 6 cases in arterial phase. In equilibrium phase, no case showed hyperattenuation, whereas isoattenuation was seen in 3 cases, and hypoattenuation in 10 cases (Table 1).

Immunostaining with anti-VEGF antibody revealed cytoplasmic reactivity in tumor cells. In arterial phase, the intensity was not correlated with the degree of enhancement on CT scan (p=0.086). On the other hand, the correlation between the attenuation value of hepatic arterial phase and the area (percentage) of positive tumor cells was statistically significant (p=0.002). No correlation was seen at equilibrium phases in either intensity (p=0.538) or area (p=0.091) of positive tumor cells in immunostaining with anti-VEGF antibody.

DISCUSSION

Dual-phase dynamic helical CT allows the evaluation of hepatic blood flow for two times, i.e., that of hepatic arterial phase followed by that of the portal venous phase or the equilibrium phase, each time with a single breath hold. Use of a dual-phase dynamic helical CT scan protocol enables improved detection of focal liver lesions because liver tumors are supplied with blood primarily from the hepatic artery (7, 8). The hepatic artery delivers about 20-25% of blood to the normal liver, whereas the portal vein delivers about 75-80% (9). After intravenous injection of a bolus of contrast material, the contrast agent is initially transported to the liver by the hepatic artery, approximately 20 sec before the opacification of

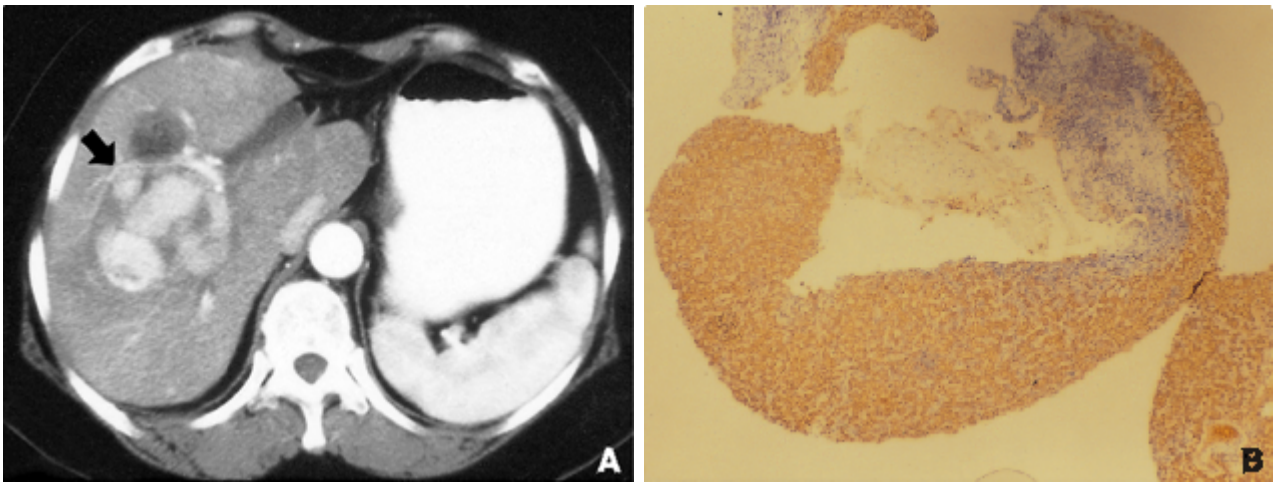

Fig. 1. Case No. 1 of 62-yr-old woman. A: Hepatocellular carcinoma with mosaic-pattern and hyperattenuation mass (arrow) is seen on CT scan in hepatic arterial phase. B: Its VEGF expression is strong (3+) and above 80% tumor cells were positively stained (immunohistochemical staining with anti-VEGF antibody, ×40).

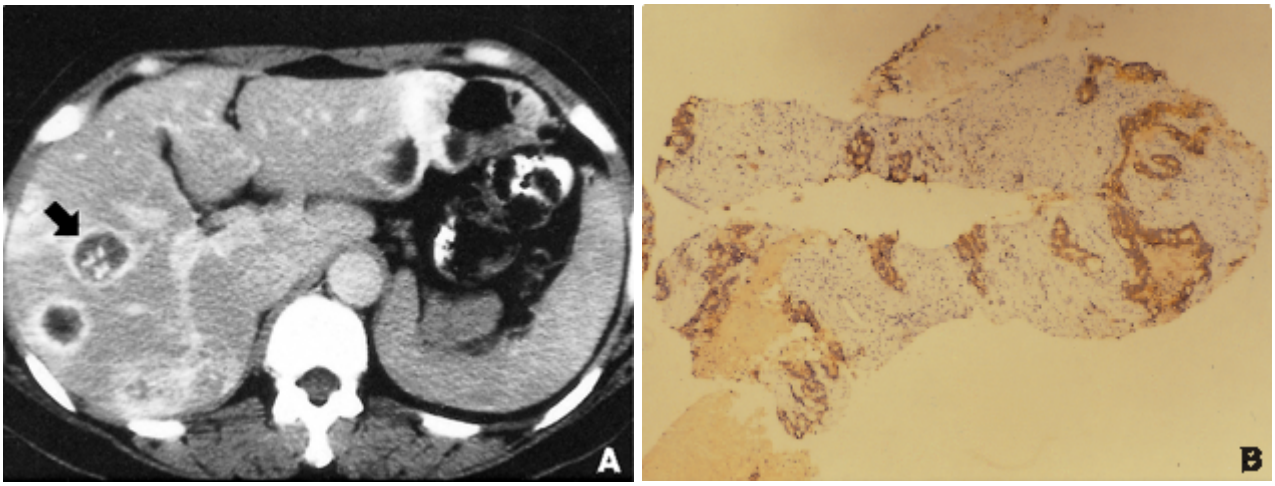

Fig. 2. Case No. 13 of 50-yr-old woman. Low attenuation mass (arrow) with rim enhancement and internal multiple dense dots on computed tomographic hepatic arteriography (A) is well correlated with sparse internal enhancement pattern (B) (immunohistochemical staining with anti-VEGF antibody, ×40).

the portal vein is achieved (10). Therefore, the arterial phase of dual-phase dynamic helical CT represents vascularity and vascular permeability of the hepatic tumors because the hepatic artery is the main blood supplier to the liver tumors (7, 8).

Water-soluble iodinated contrast agents do not readily bind to plasma protein (<5%). After intravenous administration, water-soluble iodinated contrast agents are rapidly distributed in the vascular and interstitial spaces (11). Therefore, it is possible to evaluate the permeability of vessels in target tissue with injection of water-soluble iodinated contrast agent. The alteration in permeability forms the basis for imaging mass lesions using CT by exploiting the accumulation of water-soluble iodinated contrast agents within mass. With advances in neuroradiologic methods, blood brain barrier (BBB) permeability and transport across the BBB can be now measured quantitatively in numerous physiological and pathological settings in human subjects (12, 13). Similarly, the attenuation in arterial phase represents vascularity and vascular permeability of a liver tumor. Therefore, the degree of enhancement in the arterial phase can be used to evaluate the pathophysiologic factors controlling the contrast enhancement of hepatic tumors at CT.

VEGF is a basic, heparin-binding, homodimeric glycoprotein of 45 kDa (14). VEGF plays a crucial role for the angiogenesis of tumors. Tumors produce ample amounts of VEGF, which stimulates the proliferation and migration of endothelial cells, thereby inducing tumor vascularization by paracrine mechanism (15, 16). VEGF is synthesized by tumor cells in vivo and accumulates in nearby blood vessels, which are its target of action. It can also increase the vascular permeability at a concentration of less than 1 nM, which is about 50,000 times lower than the effective concentration of histamine (17-19). Kumar et al. (20) compared the effect of various angiogenic factors (basic fibroblast growth factor, VEGF, platelet-derived growth factor, hepatocyte growth factor, and interleukin-8) on the permeability of endothelial cell monolayers in vitro and found only VEGF caused a significant increase in the permeability. Angiogenic factors other than VEGF do not cause significant vascular leakiness (19-23). All these studies about VEGF suggest that VEGF is the major factor controlling tumor vascularity and vascular permeability, and directly affects contrast enhancement of hepatic CT.

All these properties suggest that VEGF expression will be well correlated with radiological enhancement by angiogenesis and increased vascular permeability. In this study, good correlation was observed between the expression area of VEGF and the enhancement pattern in the arterial phase. By increasing vascularity and vascular permeability, VEGF may promote enhancement of hepatic tumor with contrast agents during arterial phase in CT. But, contrary to the area, the intensity was not correlated with the degree of enhancement. This result is presumably caused by difference of spatial resolution between light microscope and CT. The voxel which is minimal unit of image in CT is too large as compared with light microscope. And the attenuation number in CT is averaged by partial volume effect. Also strictly speaking, because the target of action of VEGF is vascular endothelial cell, the expression of VEGF at tumor cellular cytoplasm does not directly mean increasing of vascularity and vascular permeability. Nevertheless, according to this study, VEGF expressed at a larger area of tumor cells may induce enhancement of hepatic tumor at CT at least.

The significant finding of this study is that the VEGF might be the major pathological factor for enhancement of radiologic imaging, and we could estimate the expression of VEGF with radiologic imaging. Another significance of this study should be mentioned in terms of antiangiogenic therapy. It was found that a neutralizing antibody against VEGF inhibited tumor growth in mice (24, 25). The information about the vascularity, vascular permeability and eventually VEGF expression in a tumor, taken as a result of enhancement pattern in the arterial phase of dual-phase dynamic helical CT, can suggest a immunotherapeutic potential in the tumor.

The limitations of this study were the insufficient number of samples and not so enough amount of each biopsy specimen to represent the characteristics of the whole tumor mass. However, this study is a prospective one. And the tumor showing enhancement mixed with hyperattenuation and hypoattenuation was excluded from the first. Biopsy specimens were well targeted and made a complete match for lesions in CT. In addition, we observed statistically close relationship between enhancement pattern and VEGF expression with a large number of surgical specimens (not published).

In conclusion, VEGF may be the factor that enhances the hepatic mass with water-soluble iodinated contrast agent in CT.

REFERENCES

1. Knopp MV, Hoffmann U, Brix G, Hawighorst H, Junkermann HJ, van Kaick G. *Fast MRI contrast medium dynamics for characterization of tumors. Experiences with functional MR-mammography. Radiologe 1995; 35: 964-72.*
2. Furman-Haran E, Margalit R, Grobgeld D, Degani H. *Dynamic contrast enhanced magnetic resonance imaging reveals stress-induced angiogenesis in MCF7 human breast tumors. Proc Natl Acad Sci USA 1996; 93: 6247-51.*

3. Krause W, Schuhmann-Gampieri G. *Pharmacokinetics of contrast media. In: Dawson P, Claub W, eds. Contrast media in practice. Berlin, Pa: Springer Verlag, 1994; 26-34.*
4. Almen T. *Relations between chemical structure, animal toxicity and clinical adverse effects of contrast media. In: Enge I, Edgren J, eds. Patient safety and adverse events in contrast medium examinations. Amsterdam, Pa: Elsevier Science, 1989; 25-56.*
5. Knopp MV, Weiss E, Sinn HP, Mattern J, Junkermann HJ, Radeleff J, Magener A, Brix G, Delorme S, Zuna I, van Kaick G. *Pathophysiologic basis of contrast enhancement in breast tumors. J Magn Reson Imaging 1999; 10: 260-6.*
6. Strugar J, Rothbart D, Harrington W, Criscuolo GR. *Vascular permeability factor in brain metastases: correlation with vasogenic brain edema and tumor angiogenesis. J Neurosurg 1994; 81: 560-6.*
7. Gryspeerdt S, Hoe LV, Marchal G, Baert AL. *Evaluation of hepatic perfusion disorders with Double-phase Spiral CT. Radiographics 1997; 17: 337-48.*
8. Bonaldi VM, Bret PM, Reinhold C, Atri M. *Hepatic CT of the liver: value of an early hepatic arterial phase. Radiology 1995; 197: 357-63.*
9. Lautt WW, Greenway CV. *Conceptual review of the hepatic vascular bed. Hepatology 1987; 7: 952-63.*
10. Baron RL. *Understanding and optimizing use of contrast material for CT of the liver. Am J Roentgenol 1994; 163: 323-31.*
11. Wolf KJ, Steidle B, Skutta T, Mutzel W. *Iopromide: clinical experience with a new nonionic contrast medium. Acta Radiol Diagn (Stockh) 1983; 24: 55-62.*
12. Groothuis DR, Vriesendorp FJ, Kupfer B, Warnke PC, Lapin GD, Kuruvilla A, Vick NA, Mikhael MA, Patlak CS. *Quantitative measurements of capillary transport in human brain tumors by computed tomography. Ann Neurol 1991; 30: 581-8.*
13. Machein MR, Kullmer J, Fiebich BL, Plate KH, Warnke PC. *Vascular endothelial growth factor expression, vascular volume, and capillary permeability in human brain tumors. Neurosurgery 1999; 44: 732-41.*
14. Ferrara N, Henzel WJ. *Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun 1989; 161: 851-8.*
15. Connolly D, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, Siegel NR, Leimgthber RM, Feder J. *Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 1989; 84: 1470-8.*
16. Obermair A, Kohlberger P, Bancher-Todesca D, Tempfer C, Sliutz G, Leodolter S, Reinthaller A, Kainz C, Bbreitenecker G, Gitsch G. *Influence of microvessel density and vascular permeability factor/vascular endothelial growth factor expression on prognosis in vulvar cancer. Gynecol Oncol 1996; 63: 204-9.*
17. Senger DR, Connolly D, Perruzzi CA. *Purification of a vascular permeability factor (VPF) from tumor cell conditioned medium. Fed Proc 1987; 46: 2102.*
18. Senger DR, Connolly DT, Van De Water L, Feder J, Dvorak HF. *Purification and NH_2-terminal amino acid sequence of guinea pig tumor-secreted vascular permeability factor. Cancer Res 1990; 50: 1774-8.*
19. Dvorak HF, Brown LF, Detmar M, Dvorak AM. *Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995; 146: 1029-39.*
20. Kumar R, Yoneda J, Fidler IJ. *Distinct biologic activities of different angiogenic molecules. In Proceeding of the 88th Annual Meeting of American Association for Cancer Research, San Diego, CA, 1997; 53 (abstr).*
21. Yuan F, Chen Y, Dellian M, Safabakhsh N, Ferrara N, Jain RK. *Time-dependent vascular regression and permeability changes in established human tumor xenografts induced by an anti-vascular endothelial growth factor/ vascular permeability factor antibody. Proc Natl Acad Sci USA 1996; 93: 14765-70.*
22. Jain RK. *The Eugene M. Landis Award Lecture 1996. Delivery of molecular and cellular medicine to solid tumors. Microcirculation 1997; 4: 1-23.*
23. Roberts WG, Hasan T. *Tumor-secreted vascular permeability factor/vascular endothelial growth factor influences photosensitizer uptake. Cancer Res 1993; 53: 153-7.*
24. Kim KJ, Winder J, Armanini M, Gillett N, Phillips HS, Ferrara N. *Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth in vivo. Nature 1993; 362: 841-4.*
25. Kondo S, Asano M, Suzuki H. *Significance of vascular endothelial growth factor/vascular permeability factor for solid tumor growth, and its inhibition by the antibody. Biochem Biophys Res Commun 1993; 194: 1234-41.*