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Correlation between magnetic resonance perfusion weighted imaging of radiation brain injury and pathology

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ABSTRACT. We used magnetic resonance perfusion weighted imaging and pathological evaluation to examine different stages of radiation-induced brain injury and to investigate the correlation between the relative cerebral blood volume (rCBV) ratio and vascular endothelial growth factor (VEGF). Thirty adult rats were randomly divided into 2 groups: control and radiation group. The control group was not subjected to irradiation. The irradiation group rats were examined by magnetic resonance imaging and magnetic resonance perfusion weighted imaging at 1, 3, 6, 9, and 12 months after radiation treatment. We measured the rCBV, mean transit time, and time to peak. Hematoxylin and eosin staining, immunohistochemical staining, and electron microscopy were performed. VEGF absorbance was evaluated by immunohistochemical staining. Compared with the control group, the differences in rCBV, mean transit time, time to peak, and VEGF absorbance after 3 months were statistically significant ($P < 0.05$). rCBV was positively correlated with VEGF ($r = 0.94$, $P < 0.05$). Magnetic resonance perfusion weighted imaging can reflect pathophysiological changes in brain tissue

after irradiation. Decreased expression of VEGF plays a critical role in the pathogenesis of radiation-induced brain injury.

Key words: Magnetic resonance perfusion weighted imaging; Radiation-induced brain injury; Vascular endothelial growth factor; Immunohistochemistry

INTRODUCTION

Brain radiation injury is a severe complication of radiotherapy, with atypically clinical symptoms during early stages. Conventional imaging is insensitive and leads to irreversible brain injury after symptoms are detected (Tsao et al., 2005; Asai and Kawamoto, 2008). Therefore, the early diagnosis of radiation brain injury is very important. We induced brain radiation injury in rats and evaluated these injuries using magnetic resonance perfusion weighted imaging (MRP) and pathological examination for early diagnosis.

MATERIAL AND METHODS

Establishment of brain radiation injury in rats

Thirty female Wistar rats weighing 200-230 g were obtained from Qingdao Medicine Institute and randomly divided into a control group and 1, 3, 6, 9, and 12 months after radiation groups, with 5 rats in each group.

Intraperitoneal anesthesia was performed using 10% chloral hydrate with 0.35 mL/100 g, and then subjected to single-whole-vertical brain irradiation with a 6-MeV electron beam using a 23EX linear accelerator (Varian, Palo Alto, CA, USA), 300 Gy/min, 3.0 x 20 cm, 100 cm source-skin distance, 1.5-cm reference dose depth, and total dose of 20 Gy.

Magnetic resonance examination

We used an HDX 3.0T MRI scanner (GE Healthcare, Little Chalfont, UK) and a phased array rat coil from Shanghai Chenguang Medical Technologies Co., Ltd. (CG-MVC22-H300-AG; Shanghai, China).

The following parameters were used: T1 weighted image (T1WI) repetition time (TR) = 400 ms, echo time (TE) = 11 ms, field-of-view (FOV) = 8 cm x 8 cm, slice thickness = 2.4 mm, interlayer distance = 0 mm. T2WI TR = 3500 ms, TE = 110 ms, FOV = 8 x 8 cm, slice thickness = 2.4 mm, interlayer distance = 0 mm. For perfusion imaging, the following parameters were used: TR = 1000 ms, TE = 15.9 ms, FOV = 8 cm x 6 cm, flip angle = 30°, matrix = 64 x 64, slice thickness = 2.4 mm, interlayer distance = 0 mm. The echo planar sequence was acquired using MRP for a total of 100 times, with 10 layers in each scanning. After the 4th scanning, a needle was placed in the femoral vein and 0.1 mmol/kg Gadolinium was injected at a rate of 1 mL/s, while 2 mL/kg normal saline () was injected at the same rate into control rats. For magnetic resonance enhanced scanning, the following parameters were used: TR = 1025 ms, TE = 24 ms, TI = 860 ms, FOV = 8 x 6 cm, slice thickness = 2.4 mm, interlayer distance = 0 mm.

Pathological examination

Rats were examined by magnetic resonance, and then the brains were removed immediately, placed under normal saline lavage, and fixed with 4% paraformaldehyde. The brain tissues of rats were dehydrated in an ascending gradient series of ethanol, cleared in xylene, and embedded in paraffin. Tissue slices were prepared and subjected to hematoxylin and eosin staining and vascular endothelial growth factor (VEGF) staining.

Figures and data analysis

Data were analyzed using Functool software. MRP perfusional color maps were obtained and treated. Six randomly selected regions with the same area (region of interest = 16-20 mm^2) were examined to detect the relative cerebral blood volume (rCBV), mean transit time (MTT), and time to peak (TTP), and average values were calculated.

The absorbance value of VEGF was determined using Image-pro plus 5.0 as the average of 3 measurements. Hematoxylin and eosin staining was used to determine the morphology, structure, density, and small structure changes of the vascular lumen and basement membrane under an electron microscope.

Statistical analysis

Data were analyzed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Values are reported as means ± SD. Groups were compared by one-way analysis of variance. Measurement data were compared using the least square differences-*t* test, while the Dunnett and T3 tests were used to detect variance non-homogeneity. A value of $P < 0.05$ was considered to indicate a significant different. Correlations between rCBV and VEGF were represented as r, which is the product moment correlation coefficient for the Pearson test, and examined using the *t*-test. A value of $P < 0.05$ was considered to indicate statistical significance.

RESULTS

MRP results

rCBV in each radiation group decreased gradually over time. There were significant differences between the 3-, 6-, 9-, and 12-month groups and the control group (Table 1 and Figure 1a-c).

Table 1. Comparison of rCBV between groups (one-way analysis of variance), intra-groups (least square differences - *t*-test).

Groups	Means ± SD	Intra-group (*vs* control)			Between groups	
		Mean difference	Standard error	P	F	P
Control	288 ± 68					
1 month	325 ± 33	-36.2	28.675	>0.05		
3 months	233 ± 28	55.2	28.675	<0.05		
6 months	213 ± 53	75.0	28.675	<0.05		
9 months	175 ± 45	113.6	28.675	<0.05		
12 months	152 ± 28	136.6	28.675	<0.05	10.60	<0.05

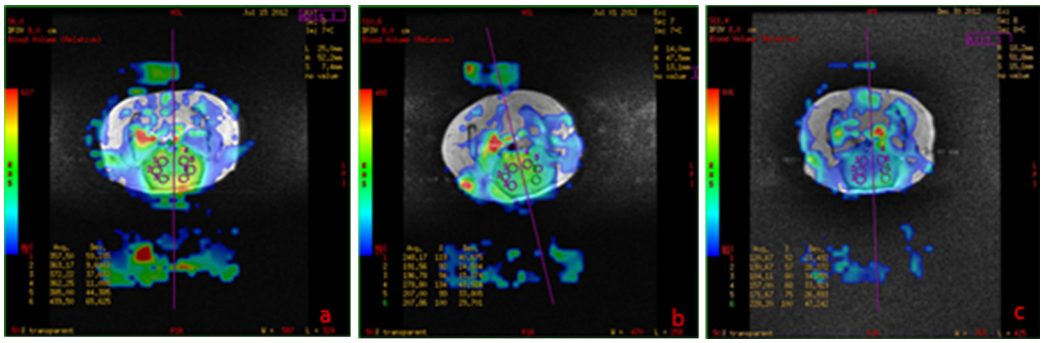


Figure 1. rCBV color maps of the control group and radiation groups: control (**a**), 3 months (**b**), and 12 months (**c**). Over time, rCBV decreased and reached the lowest level at 12 months, while the color maps changed from yellowish green to blue.

Table 2 shows that MTT clearly increased gradually over time. There were significant differences between the 3-, 6-, 9-, and 12-month groups and the control group.

Table 2. MTT value and comparison between groups (one-way analysis of variance), intra-groups (least square differences - *t*-test).

Groups	Means ± SD	Intra-group (*vs* control)			Between groups	
		Mean difference	Standard error	P	F	P
Control	3.98 ± 0.68					
1 month	4.08 ± 0.46	-0.1	1.169	>0.05		
3 months	6.70 ± 0.92	-2.712	1.169	<0.05		
6 months	6.65 ± 2.06	-2.462	1.169	<0.05		
9 months	9.62 ± 2.36	-5.636	1.169	<0.05		
12 months	11.75 ± 3.02	-7.764	1.169	<0.05	13.8	<0.05

Table 3 shows that TPP clearly increased gradually over time. There were significant differences between the 3-, 6-, 9-, and 12-month groups and the control group.

Table 3. TPP value and comparison between groups (one-way analysis of variance), intra-groups (least square differences - *t*-test).

Groups	Means ± SD	Intra-group (*vs* control)			Between groups	
		Mean difference	Standard error	P	F	P
Control	6.85 ± 2.23					
1 month	8.79 ± 1.01	-1.934	4.347	>0.05		
3 months	17.31 ± 3.90	-10.454	4.347	<0.05		
6 months	16.70 ± 2.91	-10.144	4.347	<0.05		
9 months	24.89 ± 10.11	-18.036	4.347	<0.05		
12 months	25.21 ± 12.31	-18.354	4.347	<0.05	6.34	<0.05

Pathological examination

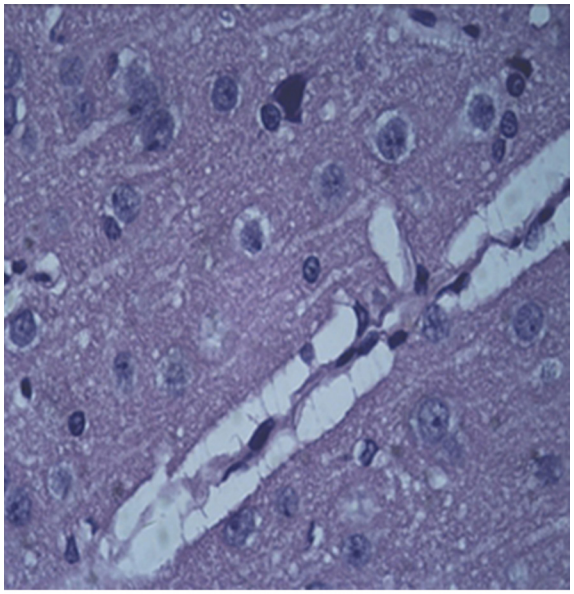
Table 4 shows that VEGF clearly decreased gradually over time. There were significant differences between the 3-, 6-, 9-, and 12-month groups and the control group.

Light microscopy analysis showed that in the control group, the vascular density was

normal and well-distributed. The vascular wall had a uniform thickness. The vessel lumen was smooth and regular. After 3 months, vascular density in the radiation groups decreased gradually, the vascular wall thickened slightly, and the vessel lumen became slightly narrower. Over time, these changes became more pronounced. Uptil 12 months, vascular density decreased, the vascular wall thickened, and the vessel lumen became narrower or blocked (Figure 2). Electron microscopy analysis revealed that in the control group, vascular density was normal and well-distributed. A number of endothelial cells were observed and these cells had complete structures. Tight junctions were observed between the cells. Over time, the basement membrane thickened gradually, endothelial cells underwent apoptosis, and the cell number decreased. Until the 12th month, the number of endothelial cells decreased, the tight junctions gradually disappeared, and organelle structures, including the mitochondria and endoplasmic reticulum, dissolved and disappeared (Figure 3).

Table 4. Absorbance value of VEGF and comparison between groups (one-way analysis of variance), intra-groups (least square differences - *t*-test).

Groups	Means ± SD	Intra-group (*vs* control)			Between groups	
		Mean difference	Standard error	P	F	P
Control	1.178 ± 0.087					
1 month	1.167 ± 0.110	0.011	0.395	>0.05		
3 months	1.003 ± 0.075	0.175	0.395	<0.05		
6 months	0.781 ± 0.044	0.397	0.395	<0.05		
9 months	0.731 ± 0.037	0.447	0.395	<0.05		
12 months	0.582 ± 0.022	0.596	0.395	<0.05	77.56	<0.05



HE staining x 400

Figure 2. Vascular wall thickened and the lumen became blocked during the 12th month.

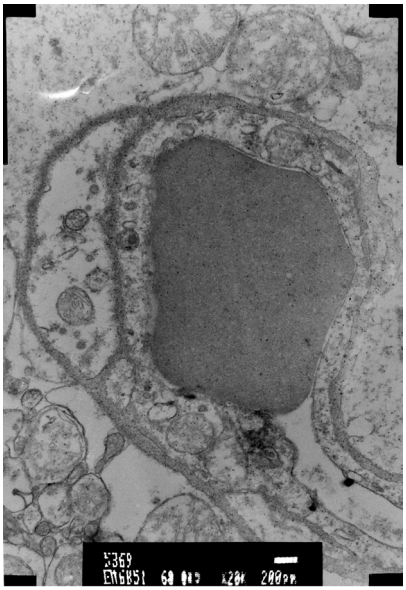


Figure 3. At the 12th month, the number of endothelial cells decreased and the basement membrane thickened and became uneven. The tight junctions between cells disappeared and organelles dissolved and disappeared.

Correlation between rCBV and VEGF

According to Pearson product moment correlation analysis, $r = 0.94$ ($P < 0.05$). There was a significant and positive linear correlation between rCBV and VEGF.

DISCUSSION

Index of MRP and immunohistochemistry detection

MRP is one of the most useful methods for diagnosing radiation brain injury. As a functional imaging technology, this method can reflect the blood perfusion status in brain tissues. Common indices used in MRP include rCBV, relative cerebral brain flow, MTT, and TTP. The value of rCBV is closely related to microvessel density, while decreased rCBV represents decreased vessel density and tissue perfusion; relative cerebral brain flow reflected blood flow in the brain tissue; MTT reflected the time required for the contrast agent to flow through the capillary; TPP was the time required for the blood to flow through a specific volume of brain tissue (Cha et al., 2002; Cha et al., 2002). Among these factors, rCBV reflected blood perfusion and is most commonly used in the clinical setting.

VEGF is the strongest cytokine promoting angiogenesis, exists in many tissues in human and animals, and plays a role in physiological regulation (Harada et al., 1994; Park et al., 1994). Our results showed that VEGF expression was closely related to microvessel density and microvasculature density. After radiation brain injury, VEGF expression and the microvessel network were altered. Currently, VEGF is the reliable marker for observing microcirculation changes in studies of radiation brain injury. The absorbance value of VEGF can be determined using a microscope camera system (Wang et al., 2006).

Correlation between MRP and pathology

Numerous studies have examined radiation brain injury using MRP. Nearly all of the results have shown that rCBV at the damage field is decreased and the values of MTT and TTP were prolonged. Bai et al. (2014) studied the effect of blood perfusion of radiation brain injury and found that most rCBV of lesions decreased compared with in the host contralateral cortex. Zhao et al. (2005) found that rCBV in the edema area of delayed radiation brain injury was decreased, while MTT and TTP were prolonged. Zhang et al. (2009) showed that during the late stage of radiation, microcirculation arterioles showed fibrinoid necrosis and the vascular wall was infiltrated by numerous plasma proteins and presented vitreous degeneration. This induced basement membrane thickening and lumen narrowing or blockage, leading to a decrease in rCBV.

Our results were consistent with those of previous studies. rCBV decreased gradually while MTT and TTP increased in the radiation groups, and the absorbance value of VEGF decreased slightly. Vascular density and structure were important factors influencing cerebral tissue perfusion, while the changes of the two induced changes in cerebral tissue perfusion. Based on the results of light microscopy, beginning in the 3rd month after radiation, vascular density in the brain tissue decreased slightly, the vascular wall thickened, and the lumen became narrower or blocked. According to the results of electron microscopy, beginning in the 3th month after radiation, endothelial cells in the brain tissue underwent apoptosis and the basement membrane thickened. Through the 12th month, the number of endothelial cells showed a large decrease, the basement membrane clearly thickened, the lumen became narrower, and organelle structures dissolved and disappeared. At the region of radiation brain injury, pathological changes that can be detected using MRP include decreased vascular density, narrowing lumen, decreased number of endothelial cells, and basement membrane thickening. Blood vessel damage caused a reduction in the blood supply at the region of radiation. Blood perfusion in the brain tissue was decreased, as revealed by the decreased in rCBV and increase in MTT and TTP. The absorbance value of VEGF in the radiation groups was slightly decreased, which was positively correlated to rCBV. These results indicate that VEGF is a key factor leading to changes in the macrostructure and microstructure of brain vessels in the region of radiation. The decrease in VEGF led to decreased vessel density and a decreased number of endothelial cells. The vessel decrease led to rCBC at the region of radiation decrease. Therefore, decreased VEGF likely plays an important role in the pathogenesis of brain radiation injury.

In conclusion, MRP reflects the pathological changes in radiation brain injury, which can be used for early diagnosis; decreased VEGF expression is the pathological basis of brain radiation injury, and this decreased expression can be detected using MRP.

Conflicts of interest

The authors declare no conflict of interest.

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