Available online at www.sciencedirect.com
SCIENCE DIRECT®
JNMU
Journal of Nanjing Medical University, 2008, 22(3):168-171
www.elsevier.com/locate/jnmu
Research Paper

Relationship among plasma endothelin, calcitonin gene-related peptide and blood flow rate of bilateral vertebral arteries in patients with cervical vertigo

Jiajun Wei*, Jinzhi Xu, Suming Zhang
Department of Neurology, the Tongji Hospital Affiliated to Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China

Received 12 November, 2007

Abstract

Objective: To investigate the relationship among plasma endothelin(ET), calcitonin gene-related peptide(CGRP) and blood flow rate of bilateral vertebral arteries in patients with cervical vertigo(CV) and to assess the effect of ET and CGRP on the onset of CV. Methods:The concentration of ET and CGRP in 64 patients with CV and 30 controls was determined by radioimmunity method. The average blood flow velocity (Vm) of bilateral vertebral arteries was detected by Transcranial Doppler(TCD). Results:Plasma concentration of ET(91.48 ± 9.08 pg/ml) and ET/CGRP ratio value(2.88 ± 0.52) in vertebrobasilar arteriospasm group were both higher than those in vertebrobasilar non-arteriospasm group and in controls, while CGRP concentration(30.66 ± 6.05 pg/ml) in vertebrobasilar arteriospasm group was lower than that in vertebrobasilar non-arteriospasm group and controls respectively. The Vm of bilateral vertebral arteries in vertebrobasilar arteriospasm group(67.97 ± 11.64 cm/s) was higher than that in vertebrobasilar non-arteriospasm group and controls respectively, having a positive correlation with ET concentration and ET/CGRP ratio value(r_1=0.52, P < 0.05; r_2=0.59, P < 0.05), but a negative correlation with CGRP concentration(r_3=-0.54, P < 0.05). There was no significant difference in ET and CGRP concentration, ET/CGRP ratio value and the Vm of bilateral vertebral arteries between vertebrobasilar non-arteriospasm group and the control group. Conclusion: All the results indicate that ET and CGRP are possibly the most important substance factors at the onset of CV with vertebrobasilar arteriospasm, and their imbalance of regulating vertebrobasilar arterial contraction and relaxation may play an important role in the onset of CV with vertebrobasilar arteriospasm.

Key words: cervical vertigo; endothelin; calcitonin gene-related peptide

INTRODUCTION

Cervical vertigo(CV) is a clinical syndrome characterized by reported affects similar to vertigo, caused by a blood-supply disturbance of cranium outer segment of vertebral artery affected by cervical pathological changes. CV often occurs with incorrect head movement[1-2], and its pathogenesis is still unknown at present. Endothelin(ET) and calcitonin gene-related peptide (CGRP) in plasma are a pair of powerful and endogenous factors maintaining arterial contraction and relaxation[3-5], which play important roles in the regulatory of cerebral blood circulation. In order to investigate the relationship among ET, CGRP and blood flow rate of bilateral vertebral arteries in patients with CV, and assess the effect of ET and CGRP on CV, we determined ET and CGRP concentration in 64 patients and 30 controls whose blood flow velocity of bilateral vertebral arteries was detected by Transcranial Doppler (TCD).

MATERIALS AND METHODS

Patients

64 patients with CV were involved in the experiment, including 30 males and 34 females, aged from 33 to 68 years old(average age was 50.7 years old), who were

*Corresponding author.
E-mail address: nicer6wei@yahoo.com.cn

hospitalized in the department of neurology during the period from August 2004 to December 2006. Referring to a relevant document[6], these patients were recruited as the following diagnostic criterions of CV:①The predominant symptom was vertigo whose onset was relevant to incorrect head movement;② Its simultaneous phenomenon might be nausea, vomiting, nystagmus, tinnitus, binocular vision disorder, conscious disturbance, drop attack, and so on, indicating insufficient blood-supply of vertebrobasilar artery; ③ The turning neck test was positive; ④ Cervical vertebra X-ray picture and CT/MRI showed that cervical spondylosis manifestations including physiological curve changes of cervical vertebra, narrowing of transverse foramen, uncovertebral joint hyperostosis, intervertebral disc herniation, and so on; ⑤ Cases with non-cervical vertigo such as otogenous vertigo and intoxication vertigo were removed. 30 healthy volunteers were chosen for the control group.

Blood flow detection of posterior cerebral circulation

Vertebrobasilar artery system blood flow was detected from occiput window by 2 MHz detecting head of TCD (TC4040 type, EME, Germany). Patients were divided into vertebrobasilar arteriospasm group(Group A) and vertebrobasilar non-arteriospasm group(Group B) according to Vm, pulsatility index(PI), resistant index(RI) and frequency spectrum manifestation of vertebrobasilar artery detected by TCD. The patients with vertebrobasilar arterial Vm > 60 cm/s and normal PI, RI and frequency spectrum were divided into the vertebrobasilar arteriospasm group(Group A)[7], and the others were assigned to the vertebrobasilar non-arteriospasm group(Group B). The average of bilateral vertebral arteries' Vm(Vm_l+Vmr/2) acted as the main parameter to evaluate the blood flow condition of posterior cerebral circulation.

Plasma samples collection

3 ml ulnar vein blood from each patient was collected after hospitalized, and every sample was added both 60 μl 10%EDTA(Sigma Company) and 80 μl Aprotinin(Sigma Company) to determine ET and CGRP concentration. These samples were centrifuged for 20 minutes(at 4℃, 3 000 r/min). The supernatant was then preserved in a refrigerator at -80℃.

Measurement of ET and CGRP

ET and CGRP concentration was determined by radioimmunity method in GC-1200 *r*-ray radioimmunity counter. Log-Logit mathematical model was applied to fit the standard curve. ET and CGRP detecting kits were provided by East Asia Radioimmunity Institute of Peking PLA General Hospital.

Statistical analysis

Analysis of variance, *q*-test and Pearson correlation analysis were performed by SPSS11.5. Differences were considered to be significant when $P < 0.05$.

RESULTS

Comparison of ET and CGRP concentration, ET/CGRP ratio value and Vm_l+Vmr/2 in different groups

According to the detecting manifestations of TCD, 31 cases(without arteriosclerosis or arteriostenosis) was used as Group A, and 33 cases(23 cases vertebrobasilar arteriosclerosis, 9 cases vertebral arteriostenosis, 1 case vertebrobasilar artery aneurysm) were designated Group B. The plasma concentration of ET, ET/CGRP ratio value and Vm_l+ Vmr/2 in Group A were both higher than those in Group B($P < 0.05$) and controls($P < 0.05$), while CGRP concentration in Group A was lower compared to Group B($P < 0.05$) and controls, respectively ($P < 0.05$). However there was no significant difference in ET and CGRP concentration, ET/CGRP ratio value and Vm_l+Vm_r/2 between Group B and the control group($P > 0.05$, Tab 1).

Relationship between Vm_l+Vm_r/2 and concentration of ET and CGRP, between Vm_l+Vm_r/2 and ET/CGRP ratio value in different groups

Vm_l+Vm_r/2 in Group A had positive correlation to ET concentration and ET/CGRP ratio value, respectively ($r_1 = 0.52$, $P < 0.05$; $r_2 = 0.59$, $P < 0.05$), while negative correlation to CGRP concentration($r_3 = -0.54$, $P < 0.05$). Vm_l+Vm_r/2 in Group B and control group had no significant correlation to the concentration of ET and CGRP and ET/CGRP ratio value, respectively ($P > 0.05$).

DISCUSSION

Being a kind of potent endogenous vasoconstrictor peptide, ET plays an important role in regulating cerebral blood flow[3,8-9]. The regional distribution of ET

Tab 1 Comparison of ET and CGRP concentration, ET/CGRP ratio value and Vm_l+Vm_r/2 in there groups

($\bar{x} \pm s$)

Groups	ET(pg/ml)	CGRP(pg/ml)	ET/CGRP ratio	Vml+Vmr/2(cm/s)
A	91.48 ± 19.08△*	30.66 ± 6.05△*	2.88 ± 0.52△*	67.97 ± 11.64△*
B	51.98 ± 9.46	47.17 ± 8.79	1.23 ± 0.33	37.96 ± 5.59
Control	46.44 ± 10.22	43.51 ± 6.26	1.13 ± 0.37	33.07 ± 2.24

compared with Group B,△ $P < 0.05$; compared with the Control, *$P < 0.05$.

receptor was widely localized in cerebral cortex, cerebellum, hippocampus, striatum and brain stem[10-11]. Our study noted that the markedly higher plasma level of ET existed in CV patients with vertebrobasilar arteriospasm. The possible reasons of higher ET level might be[4]:① When patients had an attack of CV, the stress reaction induced vascular endothelial cells to release ET; ② Various kinds of cervical pathological changes and the incorrect head movement pressed cranium outer segment of vertebral artery, which stimulated vascular endothelial cells to secrete ET; ③ The higher ET level caused brain tissue ischemia and hypoxia, which made brain vessels secrete much more ET into blood flow. In addition, our study still displayed that the average of bilateral vertebral arteries' Vm in the vertebrobasilar arteriospasm group was positively related to ET concentration significantly. These results indicate that a higher ET level may be an important endogenous factor of accelerating posterior cerebral circulation in CV patients with vertebrobasilar arteriospasm.

As a powerful vasodilator[5,12], CGRP's nerve fiber has been detected in nearly all the vascular beds. The distribution of CGRP receptor is abundant in the brain[13]. At present, CGRP's function of regulating vasomotoricity includes:①direct vasodilatation, ②antagonism of ET,③inhibition of extracellular Ca^{2+} inflow, ④mediating nitric oxide to involve in cerebral vasodilation [14,15]. In our study, we found that plasma CGRP levels in CV patients with vertebrobasilar arteriospasm was lower than that in both vertebrobasilar non-arteriospasm group and controls, and was negatively related to the average of bilateral vertebral arteries'Vm. These results indicated that lower CGRP level might be another important factor in the onset of CV with vertebrobasilar arteriospasm.

In normal physiological conditions, ET and CGRP levels keep relatively homeostatic. Our study noted that ET/CGRP ratio value in vertebrobasilar arteriospasm patients was remarkably higher than that in vertebrobasilar non-arteriospasm patients and controls, respectively. It seemed that the normal homeostasis of ET and CGRP levels was destroyed in CV patients with vertebrobasilar arteriospasm. In terms of our study results, the possible pathogenesis of CV with vertebrobasilar arteriospasm is listed below. On one hand, the combining of ET at a higher level with endothelin receptor A in vascular smooth muscle leads posterior cerebral vessel to be constricted [16], which induces ischemia and hypoxia in posterior cerebral circulation. The ischemic and hypoxic condition of the posterior cerebral circulation system then evokes an attack of vertigo. At the same time, this condition promotes vascular endothelial cells to produce and release more ET into blood flow [16], which aggravates the vertigo symptom to form a vicious cycle. On the other hand, the lower plasma CGRP weakens the function of regulating cerebral blood flow, and can not counteract the contractile function of higher ET level. Thus, the blood supply disturbance of posterior cerebral circulation becomes even worse, which further aggravates the vertigo symptom.

Some animal experiments indicated that ET A receptor antagonists such as SB234551, BSF-208075 and BQ-123 might be used to treat cerebrovascular diseases(e.g., cerebral infarction, subarachnoid hemorrhage)[17-20]. Since the normal homeostasis of ET and CGRP levels is destroyed in CV patients with vertebrobasilar arteriospasm, whether we can treat the patients with these exogenous ET A receptor antagonists and CGRP or not deserves to be further investigated.

ET and CGRP concentration, ET/CGRP ratio value and the average of bilateral vertebral arteries' Vm between non-vertebrobasilar arteriospasm group and the control group had no significant difference. This result indicated that ET and CGRP might not participate in the onset of CV with vertebrobasilar non-arteriospasm whose pathogenesis requires further study to be elucidated.

All the results indicate that plasma ET and CGRP are possibly the important substance factors at the onset of CV with vertebrobasilar arteriospasm, and their imbalance in regulating vertebrobasilar arterial contraction and relaxation may play an important role in the onset of CV with vertebrobasilar arteriospasm. Determining plasma ET and CGRP concentration in clinic may be important to understand and ultimately treat the onset of CV with vertebrobasilar arteriospasm.

References

[1] Ogino M, Kawamoto T, Asakuno K, Maeda Y, Kim P. Proper management of the rotational vertebral artery occlusion secondary to spondylosis. *Clin Neurol Neurosurg* 2001; 103:250-3.

[2] Brandt T, Bronstein A M. Cervical vertigo. *J Neurol Neurosurg Psychiatry* 2001; 71:8-12.

[3] Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, **et al**. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; 332:411-5.

[4] Ziv I, Fleminger G, Djaldetti R, Achiron A, Melamed E, Sokolovsky M. Increased plasma endothelin-1 in acute ischemic stroke. *Stroke*1992; 23:1014-6.

[5] Tan KK, Brown MJ, Hargreaves RJ, Shepheard SL, Cook DA, Hill RG. Calcitonin gene-related peptide as an endogenous vasodilator: immunoblockade studies in vivo with an anti-calcitonin gene-related peptide monoclonal antibody and its Fab' fragment. *Clin Sci* 1995; 89:565-73.

[6] Whisnant JP, Bertnstein EF, Cooper ES. Classification of cerebrovascular diseases Ⅲ. *Stroke*1990; 21: 637-76.

[7] Sloan MA, Burch CM, Wozniak MA, Rothman MI, Rigamonti D, Permutt T, **et al**. Transcranial Doppler detection of vertebrobasilar

vasospasm following subarachnoid hemorrhage. *Stroke* 1994; 25: 2187-97.

[8] Gupta YK, Briyal S, Sharma U, Jagannathan NR, Gulati A. Effect of endothelin antagonist (TAK-044) on cerebral ischemic volume, oxidative stress markers and neurobehavioral parameters in the middle cerebral artery occlusion model of stroke in rats. *Life Sci* 2005; 77:15-27.

[9] Fernández N, Monge L, García JL, García-Villalón AL, Gómez B, Diéguez G. In vivo and in vitro action of endothelin-1 on goat cerebrovascular bed. *Eur J Pharmacol* 1998; 348:199-211.

[10] Bolger GT, Berry R, Jaramillo J. Regional and subcellular distribution of [125I]endothelin binding sites in rat brain. *Brain Res Bull* 1992; 28:789-97.

[11] Narayanan U, Weiss HR, Liu X, Chi OZ. Exogenous endothelin-1 improves microvascular oxygen balance during focal cerebral ischemia in the rat. *Regul Pept* 2002; 105:1-7.

[12] Kobari M, Fukuuchi Y, Tomita M, Tanahashi N, Takeda H, Yokoyama M. Calcitonin gene-related peptide (CGRP) and the regulation of cerebral parenchymal vessels. *Brain Res* 1995; 698: 95-9.

[13] Wimalawansa SJ, el-Kholy AA Comparative study of distribution and biochemical characterization of brain calcitonin gene-related peptide receptors in five different species. *Neuroscience* 1993; 54: 513-9.

[14] Kitazono T, Heistad DD, Faraci FM. Role of ATP-sensitive K+ channels in CGRP-induced dilatation of basilar artery in vivo. *Am J Physiol* 1993; 265: 581-5.

[15] Rosenblum WI, Shimizu T, Nelson GH. Endotheliu m-dependent effects of substance P and calcitonin gene-related peptide on mouse pial arterioles. *Stroke* 1993; 24:1043-8.

[16] Park L, Thornhill J. Hypoxic modulation of striatal lesions induced by administration of endothelin-1. *Brain Res* 2000; 883:51-9.

[17] Zhang Y, Belayev L, Zhao W, Irving EA, Busto R, Ginsberg MD.A selective endothelin ET(A) receptor antagonist, SB 234551, improves cerebral perfusion following permanent focal cerebral ischemia in rats. *Brain Res* 2005; 1045:150-6.

[18] Spatz M, Yasuma Y, Strasser A, McCarron R M. Cerebral postischemic hypoperfusion is mediated by ETA receptors. *Brain Res* 1996; 726: 242-6.

[19] Hauck EF, Hoffmann JF, Heimann A, Kempski O. EndothelinA receptor antagonist BSF-208075 causes immune modulation and neuroprotection after stroke in gerbils. *Brain Res* 2007; 1157:138-45.

[20] Martine C, Hiroshi W. BQ-123, a peptidic endothelin ET_A receptor antagonist, prevents the early cerebral vasospasm following subarachnoid hemorrhage after intracisternal but not intravenous injection. *Life Sci* 1993;52:825-34.