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The role of vascular endothelial growth factor and vascular endothelial growth inhibitor in clinical outcome of traumatic brain injury

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ABSTRACT

Objectives: Tumor necrosis factor superfamily-15 (TNFSF15) also known as vascular endothelial growth inhibitor (VEGI) is a cytokine that modulates anti-angiogenesis and inflammation. Vascular endothelial growth factor (VEGF) promotes angiogenesis and vascular permeability following traumatic brain injury (TBI). The balance of VEGF and VEGI may play a key role in the maintenance of vascular and immune system homeostasis in the brain. However, the dynamic changes of circulating VEGF and VEGI after traumatic brain injury (TBI) and the correlation between plasma VEGF and plasma VEGI remains obscure. In this study, we were to investigate whether circulating VEGF and VEGI can be used as prognostic markers for patients with TBI.

Patients and methods: A prospective clinical study was conducted in two neurosurgical intensive care units of Tianjin Medical University General Hospital and Tianjin Huanhu Hospital (Tianjin, China). 40 patients and 30 healthy controls were recruited. The recruited subjects were aged over 18 with randomized gender and GCS. 1 mL of blood was withdrawn on 1, 4, 7, 14, and 21 days after TBI. Blood samples were centrifuged at 3000 rpm and the supernatants were used to measure VEGF and VEGI by ELISA kit.

Results: 1) Circulating VEGF in TBI patients was decreased on the 1st day after TBI, then climbed up on the 4th day, reaching a maximum level on the14th day after TBI, as compared to normal controls. VEGF level returned to normal level on 21th day after TBI. 2) Circulating VEGI in TBI patients was decreased on the 1st and 4th day after TBI, then climbed up on the 7th day after TBI, reaching a maximum level on 14th day after TBI, as compared to normal controls. VEGI levels declined to normal level on 21th day after TBI. 3) There was a significant positive correlation between circulating VEGF and VEGI. 4) However, TBI patients whose conditions had improved exhibited lower VEGF levels 7 days after TBI when compared to TBI patients whose condition had deteriorated. Survivors exhibited higher VEGI levels 7 days after TBI when compared to non-survivors. 5)TBI patients whose condition had improved exhibited higher VEGI levels when compared to TBI patients whose condition had deteriorated 21 days after TBI. Patients with mild TBI exhibited higher VEGI levels than those with moderate and severe TBI 21 days after TBI. 6) A lower rate of recovery and higher hospital mortality were found in patients with VEGF/VEGI ratio $\geq$ 2.366 as compared to those with VEGF/VEGI ratio < 2.366 7 days after TBI.

Conclusions: 1) VEGF level positively correlates with VEGI after TBI. 2) The elevation of VEGF exhibits an adverse effect from 4 to 14 days after TBI while it has an advantageous effect from 14 to 21 days after TBI. Increasing VEGI levels are beneficial in recovery after TBI. Controlling the ratio of VEGF/VEGI may benefit the clinical outcome following TBI.

1. Introduction

Tumor necrosis factor superfamily-15 (TNFSF15), also known as vascular endothelial growth inhibitor (VEGI) is a unique cytokine that modulates vascular homeostasis and inflammation. VEGI is highly expressed in established vasculature and is reduced at sites

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of neovascularization [1]. VEGI exhibit its anti-angiogenesis activity by inhibiting endothelial cell proliferation and endothelial progenitor cell (EPC) differentiation as well as inducing apoptosis of differentiated EPCs [2]. This anti-angiogenesis activity of VEGI resulted in inhibition of microgliosis, reduction of blood–brain barrier (BBB) leakage and parenchymal infiltration of plasma fibrinogen, as well as conferring significant neuroprotection [3].

Vascular endothelial growth factor (VEGF) signaling represents a critical rate-limiting step in physiological angiogenesis. This angiogenic response is mediated by two related receptor tyrosine kinases (RTKs), VEGFR-1 and VEGFR-2 [4]. VEGF promotes angiogenesis and repair following brain trauma [5]. VEGF is also known as vascular permeability factor, based on its ability to induce vascular leakage [6]. Consistent with a role in the regulation of vascular permeability, VEGF induces endothelial fenestration in some vascular beds and promotes growth of vascular endothelial cells (ECs) derived from arteries,veins and lymphatics [7,8]. VEGF up-regulation has been implicated to be involved in the breakdown of the BBB, thus promoting the development of brain edema.

Although VEGF itself is not inflammatory, it may modulate immune responses in the central nervous system (CNS) by opening the BBB, altering the immune-privileged status of the brain, and allowing contact between normally sequestered CNS antigens and blood–borne immune mediators [9]. VEGF mediated inflammation in the brain is characterized by upregulation of intercellular adhesion molecule-1 and the chemokine macrophage inflammatory protein-1α, as well as a preferential extravasation of monocytes [10]. Furthermore, VEGF delivery to adult mice inhibits dendritic cell development and increases production of B cells. It promotes monocyte chemotaxis and induces colony formation by mature subsets of granulocyte-macrophage progenitor cells [4].

VEGF and VEGI have opposite functions. VEGI expression is down-regulated by VEGF. Cyclo-VEGI is a competitive antagonist of VEGF, acting at both VEGFR-1 and VEGFR-2 [3,11]. Therefore, VEGI plays a role as VEGF inhibitor. The balance of VEGF and VEGI may play a key role in the maintenance of vascular and immune system homeostasis in the brain. Enhanced levels of VEGF and its receptors have been reported in the rat brain after induction of focal cerebral ischemia [12]. VEGF antagonism has beneficial effects in a mouse model of cortical ischemia, resulting in a significant reduction in the volume of the edematous tissue after the onset of ischemia in the infarct size several weeks later [13]. VEGF receptor antagonist Cyclo-VEGI, which reduces inflammatory reactivity and vascular leakiness, is neuroprotective against acute excitotoxic striatal insult [3]. However, the dynamic changes of circulating VEGF and VEGI after traumatic brain injury (TBI) and the correlation between plasma VEGF and plasma VEGI in TBI patients remains obscure. In this study, we tested the changes of VEGF and VEGI level in TBI patients and investigated whether circulating VEGF and VEGI can be used as prognostic markers for patients with TBI.

2. Patients and methods

2.1. Sample collection

40 patients and 30 healthy controls were recruited for the study. The demographic information of TBI patients and healthy control is shown in Table 1. A total of 40 patients were recruited into the statistical analysis. The baseline data of the 40 TBI patients is shown in Table 2. Experimental blood samples were collected from TBI patients aged over 18 with randomized gender and GCS who had been admitted to Tianjin General Hospital and Tianjin Huanhu Hospital from August 2013 to November 2013. The control blood samples were collected from the healthy volunteers from the health examination department of Tianjin General Hospital. The study was approved by the Institutional Review Board of Tianjin Medical University. All patients or guardians signed consent forms before enrollment.

The inclusion criteria were as below: 1) human subjects with TBI; 2) Admitted within 24 h after TBI; 3) abnormal findings under CT scan. The exclusion criteria include: 1) patients with complex trauma involving body trunk and limbs; 2) patients with hematologic disorders; 3) patients with and cancer; 4) patients on sedation.

2.2. Neurological functional outcome measurement

GCS score was employed to demonstrate the severity of neurological deficits at 1, 4, 7, 14 and 21 days after TBI. When admitted, TBI patients were separated into mild (GCS score of 13–15), moderate (GCS score of 9–12) and severe (GCS score of ≤8) injury. Improvement was defined as an unchanged GCS score of 15 or an increase ≥ 1 point during the follow-up period [14]. Deterioration was defined as decrease ≥ 1 point during the follow-up period [14].

All patients admitted were treated according to the international guidelines for TBI treatment. We did not administer any medicine that affects circulating VEGF and VEGI during the follow-up. Of the 40 enrolled TBI patients, 34 patients survived and 6 patients died during the follow-up period of 21 days. Among survival patients, the clinical conditions were improved in 26 patients and deteriorated in 8 patients.

2.3. VEGF and VEGI measurement

The fasting venous blood (1 mL) were collected in the morning with EDTA tube (0.5 mM final concentration). Circulating Treg cells was detected on 1, 4, 7, 14, and 21 days after TBI. Blood samples were also collected from healthy subjects to obtain the normal reference values. Blood samples were centrifuged at 3000 rpm and the supernatants were stored at −80 °C.

VEGF and VEGI ELISA (enzyme-linked immunosorbent assay) kits were used to identify plasma VEGF and VEGI levels (Abcam, Cambridge, Massachusetts). 100 μL of standard protein or sample was loaded per well of the 96 well microplate. The microplate was

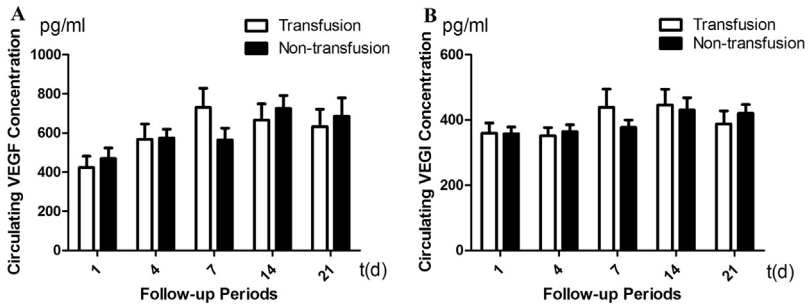

Fig. 1. Blood transfusion exerted on effect on the levels of circulating VEGF (A) and circulating VEGI (B) in TBI patients.