Hepcidin Levels Are Increased in Patients with Acute Ischemic Stroke: Preliminary Report

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Background: Our current understanding of iron balance in acute ischemic stroke (AIS) is still limited. The objective of this study was to evaluate levels of iron homeostasis proteins–hepcidin (25–amino acid form) and soluble hemojuvelin (sHJV) together with hepcidin/sHJV ratio (Hepc/sHJV) and soluble transferrin receptor (sTfR) in patients with AIS. In addition, the effect of timing of blood collection, type of stroke treatment, and scores on the National Institutes of Health Stroke Scale were investigated. *Methods:* Participants comprised 31 patients diagnosed with AIS and 20 matched healthy controls. Venous blood samples were drawn on the first day and on the seventh day after stroke onset. Individuals who had experienced a stroke were subdivided according to type of treatment (thrombolysis group, n = 12 versus nonthrombolysis group, n = 19). Plasma hepcidin, sHJV, and sTfR levels were determined by the enzyme-linked immunosorbent assay method. *Results:* We found that plasma hepcidin levels were significantly higher in ischemic stroke patients compared with the control group (median, 19.82 versus 12.62 ng/mL, $P = .04$). Furthermore, levels of hepcidin on the seventh day (1 week after diagnosis) were significantly higher in patients treated with thrombolysis than in patients not treated with thrombolysis (median, 22.16 versus 16.21 ng/mL, $P = .04$). *Conclusions:* The study provides evidence that AIS is associated with increased hepcidin levels. Stroke treatment may have an influence on hepcidin synthesis. **Key Words:** Stroke—hepcidin—hemojuvelin—heparin.

Over the last decade, the number of proteins involved in iron homeostasis has been described. The paramount role in iron metabolism is played by hepcidin, a cysteine-rich peptide, which is synthesized and is secreted principally by the hepatocytes. Hepcidin degrades its receptor, the sole cellular iron exporter ferroportin, and inhibits duodenal iron absorption as well as blocking macrophage iron efflux, resulting in hypoferremia. Hepcidin levels are regulated by inflammatory stimuli, iron stores, erythropoietic activity, and hypoxia.[1] Indeed, hepcidin synthesis is sophisticatedly controlled via the bone morphogenetic proteins (BMPs) pathway. Hemojuvelin (HJV, also known as repulsive guidance molecule c), a glycophosphatidylinositol-linked membrane protein, is the BMP coreceptor[2] that binds BMP6 and increases hepcidin expression and reduces serum iron.[3] In addition, HJV can be released from cells and exists in blood as a soluble hemojuvelin (sHJV). In contrast to the membrane-associated form, a soluble form of HJV inhibits hepcidin messenger RNA (mRNA) expression.[4,5] Nevertheless, only few data are available in the literature on the participation of hepcidin–HJV axis in the balance of iron in humans.

Stroke is a leading cause of mortality and a prominent health problem worldwide. As reported by the American Heart Association, roughly 87% of all stroke cases are ischemic.[6] In Poland, about 80% of strokes are caused by ischemia.[7] Anemia has been proposed as a meaningful factor in the pathophysiology of ischemic stroke.[8,9]

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Furthermore, in recent years, there has been considerable interest in the association of iron metabolism and stroke. Clinical studies have concluded that iron deficiency anemia is a risk factor for ischemic stroke in adults[10] likewise in children.[11]

Despite the growing evidence of iron imbalance in ischemic stroke, hepcidin and sHJV levels in the blood of those patients have not yet been researched. The purpose of this study was 2-fold. The objective was to evaluate the levels of iron homeostasis proteins (hepcidin, sHJV, and soluble transferrin receptor [sTfR]) in patients experiencing acute ischemic stroke (AIS). The second was to assess the relationship between laboratory data and type of treatment for ischemic stroke. The asset of our study is that we investigated the potential effects of stroke therapy and the timing of blood sampling on the levels of proteins involved in iron balance.

Materials and Methods

Patients

In this study, 31 patients aged older than 18 years who had a current diagnosis of AIS were recruited between 2011 and 2013. The clinical diagnosis of AIS was based on the World Health Organization criteria.[12] Computed tomography was used to further confirm the diagnosis of stroke. At admission and at 1 week after the onset of stroke, the neurologic deficit was quantified using the National Institutes of Health Stroke Scale (NIHSS). Ischemic stroke was subtyped according to the Oxfordshire Community Stroke Project classification. Demographic and clinical data including age, gender, drug use, and history of risk factors (systolic and diastolic blood pressure, nicotine consumption, and diabetes mellitus) were recorded. Diabetes mellitus was defined as having one of the following: insulin or oral hypoglycemic agents or fasting blood glucose of 7 mmol/L or more (≥126 mg/dL) on 2 occasions. According to the American Heart Association, hypertension was defined as a systolic blood pressure of 140 mm Hg or more or a diastolic blood pressure of 90 mm Hg or more or the patient was on an antihypertensive drug. Current cigarette smokers were defined as those reporting smoking before stroke onset. Two patients had a previous history of stroke, and 3 participants had a previous history of myocardial infarction. Patients were excluded from the study if they had (1) hemorrhagic stroke, (2) transient ischemic attack (symptoms duration <24 hours), (3) systemic malignancy, and (4) renal dysfunction. On the grounds that hepcidin and HJV are both essentially synthesized by hepatocytes, stroke patients with any liver dysfunctions were also deemed ineligible. In addition, a complete blood count was performed at the time of initial diagnosis as the part of routine practice. Therefore, only patients with normal levels of hemoglobin were included in this study. The median C-reactive protein levels in patients with ischemic stroke were 3.35 mg/L (the laboratory analyzer AU680 Chemistry System; Beckmann Coulter, Brea, USA).

All stroke subjects were clustered into 2 groups depending on the criteria for intravenous alteplase administration (recombinant tissue plasminogen activator [rtPA], *alteplasum*, Actilyse 50; Boehringer Ingelheim, Ingelheim, Germany)[13]: (1) patients receiving rtPA at .9 mg/kg (maximum 90 mg) within 4.5 hours of the stroke onset (n = 12) and (2) patients not treated with thrombolysis (n = 19). To outline further, the first group contains 7 patients treated by rtPA only and 5 patients who have received rtPA in combination with subcutaneous enoxaparin sodium, which is a low-molecular-weight heparin derivative (LMWH, *Enoxaparinum natricum*, Clexane; Sanofi–Aventis, Paris, France). Enoxaparin was administered at a dose of 60 mg twice daily from the second day onward during the patients' stay in the hospital. Of the 19 patients in the second group, 11 patients received enoxaparin sodium given by subcutaneous injection, whereas the remaining 8 patients received oral antiplatelet drugs. A dose of 40 mg (4 patients), 60 mg (4 patients), 80 mg (2 patients), or 100 mg (1 patient) of enoxaparin once or twice per day was administered in the 11 individuals who were not treated with thrombolysis.

Twenty healthy age- and sex-matched control volunteers with a negative history of stroke and without any laboratory and clinical findings of anemia were recruited by the Neurological Outpatient Clinic in Jan Biziel University Hospital No 2, Bydgoszcz, Poland during the same period as the stroke patients. In the control population aged from 33 to 67 years, there were 9 women and 11 men. The study was approved by the Regional Ethical Committee of Collegium Medicum of Nicolaus Copernicus University.

Blood

Peripheral venous blood samples were obtained from each stroke patient 2 times: (1) at the time of diagnosis (first day) and (2) 1 week after diagnosis (seventh day). Blood from patients treated intravenously with rtPA was drawn 2 hours after drug administration. Patients who did not receive rtPA underwent a venous blood draw during the first 2 hours after stroke diagnosis. Blood was collected into .109-M sodium citrate (a final blood-to-anticoagulant ratio, 9:1) using Vacutainer tubes (Becton Dickinson, Franklin Lakes, USA). After being centrifuged at 1500*g* for 20 minutes (+4°C), the nonhemolyzed plasma samples were placed in 200 μL portions and frozen at −80°C until analysis. Moreover, plasma specimens showing macroscopic evidence of lipemia were excluded. Fasting control blood samples were collected from healthy subjects only once. Plasma samples of controls were prepared in the same way as in the group of stroke patients. No repeated freeze–thaw cycles were performed before analysis.