Iron-Related Damage in Acute Ischemic Stroke

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Background and Purpose Although iron-mediated mechanisms are important in experimental brain injury after carotid occlusion, their clinical role in acute ischemic stroke has not been determined. We evaluated the influence of iron stores, measured as serum ferritin, on the outcome of acute cerebral infarct.

Methods Admission and fasting glycemia, glycosylated hemoglobin, serum cortisol, serum ferritin, and 24-hour urinary free cortisol levels were measured on the first day of hospitalization in 67 patients admitted with an acute ischemic stroke of less than 24 hours' duration. Patients were classified into two groups according to their Canadian Stroke Scale (CSS) score on day 30: good outcome group (alive and CSS score >7 points) and poor outcome group (dead or CSS score ≤7 points).

Results Thirty-three patients (49%) had good outcome and 34 (51%) poor outcome. Fasting glycemia (P=.001), serum cortisol (P<.001), and urinary free cortisol (P=.001) but not admission glycemia and glycosylated hemoglobin had higher levels in patients with poor outcome. Serum ferritin values were greater in the poor outcome group (218±156 µg/L versus 133±125 µg/L; P=.004), and a correlation between ferritin values and degree of worsening or improvement of the CSS score on day 30 was found (P=.002). Serum cortisol (odds ratio [OR] 6.7; 95% confidence interval [CI], 1.7 to 26), fasting glycemia (OR, 5.4; 95% CI, 1.2 to 24), and serum ferritin (OR, 4.6; 95% CI, 1.1 to 19) were independently related to poor outcome in a logistic regression analysis.

Conclusions High serum ferritin levels within the first 24 hours of hospitalization for an acute ischemic stroke are related to a poor prognosis, independent of the stress response. More research is needed to determine the origin and increased serum ferritin levels and the therapeutic implications.

Key Words • glucose • iron • prognosis • stroke outcome

The involvement of free radicals in central nervous system ischemia is under intensive study. Free radicals can react with and damage proteins, nucleic acids, and lipids, particularly the fatty acid component of membrane phospholipids, producing changes in the fluidity and permeability of the cellular membranes. The consequences of these alterations are potentially lethal for cellular function.1 Since unlike most extracellular fluids the cerebrospinal fluid has low concentrations of ferritin-binding proteins, iron released from damaged brain cells during ischemia is more likely to catalyze the generation of radical hydroxyl, the more malignant free-radical species. The superoxide dismutase enzyme scavenges this hydroxyl radical and inhibits iron release from intracellular stores such as ferritin. However, the central nervous system is relatively poorly endowed with superoxide dismutase, one of the mechanisms of cell defense against free radicals.2

In experimental models the inhibitors of iron-induced lipid peroxidation (lazaroids) attenuated 24-hour post-ischemic cortical neuronal necrosis after 3-hour unilateral carotid occlusion.3 In a recent study gerbils fed with a low-iron diet for 8 weeks had decreased brain and serum iron levels, fewer neurological deficits, and decreased brain edema after temporary unilateral carotid ligation and reperfusion.4 Thus, ferrous iron-mediated free-radical mechanisms may be important in ischemic acute stroke, but their clinical role has not been determined. The aim of our study was to determine the influence of iron, measured as serum ferritin, on the outcome of ischemic acute stroke.

Subjects and Methods From October 1990 to January 1992 we studied prospectively 118 consecutive patients with acute stroke of less than 24 hours' duration to test nutritional status, glucose profile, and stress response. Exclusion criteria were (1) age older than 80 years; (2) recovery of symptoms before entry; (3) liver disease, renal failure, thyroid dysfunction, past or current inflammatory diseases, hematologic malignancies, solid tumors, and medical conditions for which death was likely within 1 year; (4) neurological deficit from a previous stroke; (5) ongoing antihypertensive treatment with ß-methyldopa, clonidine, and ß-blockers; (6) therapy with benzodiazepines or neuroleptics; (7) failure to obtain informed consent to participate; and (8) unfilled protocol conditions. For the purpose of this study we excluded 30 patients with cerebral hemorrhage and 21 patients in whom stored frozen blood samples to measure serum ferritin were not available.

Laboratory parameters used in this investigation were glycemia levels at admission before administration of intravenous fluids; fasting glycemia, glycosylated hemoglobin, ferritin, and serum cortisol within the first 24 hours of hospitalization; and free cortisol in urine collected during the first day. Serum ferritin was determined by microparticle enzyme immunoassay (IMX system, Abbott Laboratories). The intra-assay and interassay coefficients of variation at the serum ferritin level of 80 µg/L were 2.7% and 3.5%, respectively. Serum HbA1 was analyzed by ion exchange chromatography in microcolumns (BioSystems), glycemia by the glucose-oxidase method, and...
serum and urinary cortisol by microparticle enzyme immunoassay (IMX system, Abbott Laboratories).

Neurological deficit was evaluated by the Canadian Stroke Scale (CSS) score at admission, daily during the first 7 days, and monthly during the 3-month monitoring period by a neurologist (A.D.). Functional capacity was determined by the modified Barthel Index on days 15, 30, and 90. Computed tomographic scans were performed on all patients during the first week to confirm the clinical diagnosis. Carotid artery ultrasonography, cerebral angiography, and echocardiography were done in several cases to clarify the cause of the cerebral infarct.

The patients were treated for associated illnesses and received early rehabilitation. Glucose infusions and corticosteroids were not prescribed. Patients were classified into two groups according to neurological deficit 1 month after the ischemic stroke: (1) good outcome (alive and CSS score >7 points) and (2) poor outcome (dead or CSS score ≤7 points).

Data were analyzed by means of BMDP-1990 programs. Depending on the normality and homogeneity of the variances, one-way ANOVA or Mann-Whitney rank-sum tests were used to compare continuous variables between groups. To determine if ferritin was an independent risk factor of poor outcome (dependent variable), we used a stepwise logistic regression analysis based on the maximum likelihood ratio, including age, history of diabetes, fasting glycemia, serum cortisol, and ferritin as covariates. Enter and remove limits were stated at 0.05 and 0.1 values, respectively. We assigned a value of 0 to good outcome and a value of 1 to poor outcome. Continuous variables were categorized (0, low; 1, high) to allow the estimation of the odds ratio (OR); cutoff values were established with the method described by Robert et al.7 The interaction between ferritin and fasting glycemia was tested in a model that included the three variables selected in the stepwise procedure. The Spearman nonparametric correlation coefficient was applied to test the relation between serum cortisol, ferritin levels, and the degree of worsening or improvement in the CSS score on day 30 with respect to the admission score calculated for each patient, following the method described in a previous report.8

Results

The mean age of the 67 patients included was 66±10 years; 61% were male. Hospitalization delay (time from onset to inclusion) was 10±6.7 hours. Diabetes mellitus was present in 22%, hypertension in 52%, heart disease in 28%, and previous transient ischemic attack or stroke in 22% of the patients. Cerebral infarct was atherothrombotic in 35 patients, lacunar in 15, and cardioembolic in 17. The carotid vascular territory was involved in 85% of the patients. At admission mean CSS score was 6.1±2.4 points. Twelve patients died during the first month of the monitoring period. Most deaths (66%) occurred within the first week of the stroke; autopsies were performed in 3 patients. Causes of death were encephalic herniation in 4 patients, thrombosis of the basilar artery in 2, pulmonary diseases in 2, sepsis in 2, and unknown in 2. The length of hospitalization was 20±17 days. On day 30 of the follow-up the mean CSS score of the survivors was 7.5±2.5, and the Barthel Index was 12±8. Thirty-three patients (49%) were classified in the good outcome group and 34 (51%) in the poor outcome group. In the 21 patients in whom serum ferritin was not measured, mortality (24%) and poor clinical outcome (46%) were similar to those of the patients included in the study.

Glucose levels under fasting conditions were significantly higher in patients with poor outcome than in patients with good outcome (Mann-Whitney, P<.001). This significant difference was even higher among the nondiabetic patients (ANOVA, P=.0004). Admission glycemia and HbA1c were not different between groups. Serum cortisol (ANOVA, P=.0002) and urinary free cortisol (Mann-Whitney, P=.001) levels were significantly greater in the group with poor outcome (Table). Ferritin was higher in the group with poor prognosis (Mann-Whitney, P=.004). Serum ferritin was also significantly higher in patients with Barthel Index lower than 12 points on day 30 of the follow-up (207±159 μg/L versus 140±124 μg/L; P=.027). There was no correlation between serum cortisol and ferritin values (Spearman correlation coefficient = .181, P=NS) (Fig 1).

We found a modest significant correlation between the ferritin values and the degree of worsening or improvement of the CSS score on day 30 of the follow-up (Spearman correlation coefficient = .365, P<.01) (Fig 2). In the logistic regression analysis, serum cortisol values of more than 0.84 nmol/L (OR, 6.7; 95% confidence interval [CI], 1.7 to 26), fasting glycemia higher than 7.2 nmol/L (OR, 5.4; 95% CI, 1.2 to 24), and serum ferritin levels of more than 190 μg/L (OR, 4.6; 95% CI, 1.1 to

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nP indicates number of patients studied; values are mean±SD. *P<.01, tP<.05.

Fig 1. Scatterplot shows serum ferritin concentration and serum cortisol levels, both determined within the first 24 hours of admission for acute ischemic stroke (Spearman correlation coefficient, r = .181, P=NS).
We did not determine total iron binding capacity between worsening or improvement of the neurological deficit as analysis, more than twice that for serum ferritin.14 Cause it also has considerable variability in laboratory and hemolysis during blood sampling and does not susceptible to measurement error caused by contamination the infarcted area. Serum iron concentration is susceptible to measuremenr error caused by contamination and hemolysis during blood sampling and does not indicate storage of iron in the body as well as ferritin.14 We did not determine total iron binding capacity because it also has considerable variability in laboratory analysis, more than twice that for serum ferritin.14

Ferritin is also a poor prognostic index in critically ill patients. Bobbio-Pallavicini et al15 reported that serum ferritin determined on the third day after surgery in 51 patients parallels the severity of the complications and related mortality and constitutes a useful marker of the severity of the patient’s clinical status, predicting the patient’s outcome. The role played by serum ferritin is not yet established, but it seems justified to consider serum ferritin as an acute-phase protein that reflects the severity of the critical illness15 and the stress response in acute stroke.16

If there is no record of ferritin values previous to acute stroke, we do not know if increased ferritin within the first hours of ischemic stroke in our patients with poorer prognosis is secondary to the stress reaction or is a reflection of the body’s iron stores. However, evidence exists against an early increase of ferritin secondary to stress response. Serum ferritin rose mainly after the first 24 hours in pharmacologically induced stress in healthy volunteers, as reported by Elín et al17; the maximum increase in serum and cerebrospinal fluid ferritin in the six patients with ischemic stroke studied by Hallgren et al13 was obtained after 96 hours; and we found no correlation between serum cortisol and serum ferritin during the first day of hospitalization in patients with acute stroke.

Admission glycemia and HbA1c in our patients support the theory that high fasting values of glucose the day after stroke were secondary to the stress response.18 However, although both serum ferritin and glycemia are elevated during stress, the weak correlation with serum cortisol and the fact that ferritin was selected as the predictive independent variable in the logistic regression analysis suggest that high ferritin levels may have a direct neurotoxic effect in ischemic acute stroke.

Oppenheimer et al15 suggested that the poorer prognosis observed in patients with acute ischemic stroke and high levels of serum glucose could depend in part on associated premorbid atherosclerosis. We consider that the high prevalence of coronary19,20 and carotid21 atherosclerotic disease recently described in patients with high serum ferritin may influence the poor outcome observed in our patients.

Discussion
Several potential therapeutic strategies in acute stroke are under clinical investigation since basic biological studies on experimental cerebral ischemia have obtained promising results.9 Recently attention has been focused on controlling hyperglycemia because it has been related to infarct size10 and progressing cerebral infarct.8,11 Lactate and unbuffered hydrogen ions produced by anaerobic glycolysis accumulate in tissue in proportion to the carbohydrate stores present at the onset of ischemia. The toxicity of hydrogen ions, especially their ability to facilitate ferrous iron-mediated free-radical injury, may be responsible for the increased tissue damage.12 Thus, hyperglycemia may potentiate the deleterious effects of iron after cerebral ischemia. Despite this experimental evidence, no clinical studies have been conducted to establish the relation between iron levels and brain damage in acute ischemic stroke. Hallgren et al13 found increased serum and cerebrospinal fluid ferritin in six patients with cerebral infarction, but they did not report the clinical outcome. Our results demonstrate that (1) on the first day of hospitalization for an acute ischemic stroke, levels of serum ferritin are significantly higher in patients with poor outcome and (2) levels of serum ferritin correlate with the degree of worsening or improvement of the neurological deficit as measured by change in CSS score. Bias by an early death or different outcome in patients in whom frozen blood samples to test ferritin were not available is unlikely because this group had the same clinical evolution as the group of patients included in the study.

Ferritin is more suitable than serum iron levels and transferrin saturation to test the availability of iron in the infarcted area. Serum iron concentration is susceptible to measurement error caused by contamination and hemolysis during blood sampling and does not indicate storage of iron in the body as well as ferritin.14 We did not determine total iron binding capacity because it also has considerable variability in laboratory analysis, more than twice that for serum ferritin.14

Fig 2. Scatterplot shows serum ferritin concentration within the first day of hospitalization and degree of worsening or improvement in the Canadian Stroke Scale score on day 30 with respect to the admission score after acute ischemic stroke (Spearman correlation coefficient, r=.365, P<.01).

19) were independently related to poor outcome. There was not a positive interaction between glucose and high ferritin serum levels. Age and history of diabetes were not selected by the model.

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