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Higher Level of Serum Heme Oxygenase-1 in Patients With Intracerebral Hemorrhage

Xin Li[1], Changqing Li[2], Lichun Hou[1], Mei He[1], Guangfu Song[3], Shiyan Ren[4], Chengwu Han[5]

[1]*Department of Neurology and* [3]*Department of Neurosurgery, First Affiliated Hospital of Jiamusi University, Helongjiang, China*

[2]*Department of Invasive Technology, Ditan Hospital, Capital Medical University, Beijing, China*

[4]*Cardiovascular Center and* [5]*Clinical Laboratory, China-Japan Friendship Hospital, Beijing, China*

The objective of this paper was to investigate the association of the serum level of heme oxygenase-1 in patients with intracerebral hemorrhage (ICH) with the risk of ICH. Heme oxygenase-1(HO-1) metabolizes heme into biliverdin, bilirubin, carbon monoxide, and iron, our recent study showed that serum level of HO-1 was increased in stroke patients, yet the association of HO-1 level with risk of intracerebral hemorrhage (ICH) is poorly known. Forty patients with ICH and another 40 patients without ICH were recruited. The serum level of HO-1, total, and direct bilirubin were measured. The level of HO-1, serum total bilirubin, and direct bilirubin, as well as blood pressure were increased in ICH group than in control group ($P < 0.001$). The level of HO-1, both systolic and diastolic blood pressure had a significant difference between subgroups ($P < 0.05$). Multivariate regression analysis showed that poor compliance to medicine for hypertension, the serum level of HO-1, and systolic blood pressure were associated with the prevalence of ICH. Blood pressure, serum HO-1, serum total bilirubin, and direct bilirubin were raised in patients with ICH who did not take medicine for hypertension compared with those who did, and increased in ICH patients in comparison with control group. Further investigation in multiple medical centers with large number of cohorts is warranted to verify these results.

Corresponding author: Shiyan Ren, MD, PhD, Vascular Surgery, China-Japan Friendship Hospital, No. 2, Yinghua East Road, Chaoyang District, PR China 100029.
Tel.: 86 13661004048; Fax: 86 10 64287998; E-mail: rens66@126.com

Corresponding author: Chengwu Han, MD, Clinical Laboratory, China-Japan Friendship Hospital, No. 2, Yinghua East Road, Chaoyang District, PR China 100029.
Tel.: 86 13911103590; Fax: 86 10 64287998; E-mail: 13911103590@163.com

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Heme oxygenase (HO) presents in several mammalian tissues, catalyzes the rate-limiting step in heme degradation, regulates heme protein turnover, and cleaves the heme ring to form biliverdin that is subsequently converted to bilirubin.[1,2,3] There are 3 known HO isoforms: HO-1, HO-2, and HO-3. HO-1, a 32-kDa heat shock protein, is inducible by several stress factors such as oxidative stress, hypoxia, heavy metals, etc. In humans, the ho-1 gene (HMOX1) is located on chromosome 22q12 and contains 4 introns and 5 exons. HO-1 enzymes have been characterized as endoplasmic reticulum associated proteins due to the abundant detection of HO activity in microsomal fractions. HO-2 is a constitutive isoform that is expressed under homeostatic conditions. Both HO-1 and HO-2 are ubiquitously expressed and catalytically active. HO-3 is not catalytically active, there are no functional HO-3 genes at least in rats.[4]

Expression of HO-1 has been described in microglia, astrocytes, and neurons in many experimental models such as subarachnoid hemorrhage, ischemia and traumatic brain injury, as well as in human neurodegenerative diseases.[1] HO-1 has been reported to be associated with prevalence of stroke.[5] Our recent study showed that HO-1 level was higher in patients with stroke than with TIA.[6] The stroke can be caused by ischemic infarction or intracerebral hemorrhage (ICH), which is one of the most prominent conditions leading to high morbidity and mortality.[7] Bilirubin, a powerful antioxidant, is induced after hemorrhagic stroke, and is significantly elevated in patients with hemorrhagic stroke.[8] The serum bilirubin level reflects the intensity of oxidative stress and HO-1 expression in response to oxidative stress in various diseases.[8] Currently, no data regarding the association of serum HO-1 level in ICH patient is available, and we hypothesize that the serum level of HO-1 in patients with ICH might increase in response to the stress condition of the ICH. Therefore, we analyzed the serum level of HO-1 in ICH patients and those without ICH. To evaluate the role of bilirubin as a marker of oxidative stress in ICH, we measured serum bilirubin concentrations as well.

Materials and Methods

Patients

From February 2012 to November 2014, consecutive 40 patients with ICH verified by computed tomography (CT) or magnetic resonance imaging (MRI) were examined in this study, another 40 patients with headache hospitalized without ICH were used as control. The study was approved by our hospital ethical committees, the methods were carried out in accordance with the approved guidelines and all participants provide their written informed consent for this study.

Inclusion conditions included patients with new ICH occurred within 7 days confirmed by image studies (CT or MRI), aged 40 to 80 years old, willingness to provide written consent for the study; exclusion criteria were patients with ischemic stroke, ICH occurred over 7 days earlier. Control group was patients with headache with no stroke or transient ischemic attack.

Blood tests and analysis

Blood samples were collected into tubes with no anticoagulants, and allowed to coagulate, then centrifuged at 4500 g, the serum was collected and stored at –20°C until measurements were performed. HO-1 (c-18, sc-1796, Santa Cruz Biotechnology, Inc, Santa Cruz, California) was detected by ELISA, and serum total bilirubin and direct bilirubin were determined by automated biochemical profiling (Beckman Synchron LX20, Beckman Coulter, Inc, Brea, California) with Diazo method.

Statistical Analysis

Group differences were tested with 1-way analysis of variance (ANOVA) followed by the post hoc Bonferroni test. Differences between 2 groups were analyzed with the Student's t test after testing for normality. Mean values were compared by the Kruskal–Wallis H-test and Mann–Whitney U test. Fisher's exact test was used for categoric data. A two-tailed P value < 0.05 was considered as statistical significance. The software SSPS 12.0 for windows (SPSS Inc, Chicago, Illinois) was used for all statistical analyses.

Results

The level of HO-1, serum total bilirubin and direct bilirubin as well as systolic and diastolic blood pressure were increased in the ICH group in comparison with that in the control group ($P <$

Table 1 Serum concentrations of HO-1, total bilirubin, direct bilirubin, systolic, and diastolic blood pressure in patients with or without intracerebral hemorrhage (ICH)

	ICH (n = 40)	non-ICH (n = 40)	Reference	*P*
Age (year)	68.5 ± 10.3	67.63 ± 13.33	_	0.74
Male (%)	27 (67.5)	28 (70.0)	_	0.81
Hypertension (%)	26 (65)	24 (60)	_	0.644
DM (%)	16 (40.0)	17 (42.5)	_	0.82
Dsylipidemia (%)	12 (30)	8 (20)	_	0.302
Smoker (%)	10 (25)	13 (32.5)	_	0.633
Alcohol drinker (%)	13 (32.5)	14 (35)	_	0.809
HO-1 (μmol/L)	204.69 ± 77.10	136.33 ± 38.87	80.0–150.0	<0.001
TBIL (μmol/L)	14.55 (9.05–18.98)	8.42 (4.1–14.5)	5.00–21.00	<0.001
DBIL (μmol/L)	5.15 (2.25–5.90)	2.1 (1.6–5.0)	0.00–7.00	<0.001
SBP (mmHg)	158.4 ± 23.8	129.7 ± 10.89	<140	<0.001
DBP (mmHg)	98 (80–98)	78 (76–88)	<80	<0.001

DM, diabetes mellitus; HO-1, heme oxygenase-1; TBIL, total bilirubin; DBIL, direct bilirubin; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Differences between 2 groups were analyzed with the Student's *t* test after testing for normality. Mean values were compared by the Kruskal–Wallis H-test and Mann–Whitney *U* test. Fisher's exact test was used for categoric data.

0.001; Table 1). Based on compliance to take medicine for hypertension, patients with ICH were further subgrouped into compliant, noncompliant, or none, implying patients on medicine regularly, irregularly or never, respectively. The level of HO-1, both systolic and diastolic blood pressure had a significant difference between subgroups ($P < 0.05$; Table 2).

Multivariate regression analysis showed that compliance to antihypertensive medication had the highest risk of ICH, followed by the HO-1, systolic blood pressure and direct bilirubin. After adjustment of age, gender, smoking, diabetes, and dyslipidemia ratio, patients with hypertension who never take medication for hypertension had 88.2 times odds of ICH in comparison with those who are on medication. Level of HO-1, and systolic blood pressure were associated with the prevalence of ICH. A 1 μmol/L increment in serum HO-1, 1 mmHg increment in systolic blood pressure and 1 μmol/L increment in serum direct bilirubin was associated with 0.96, 0.93, and 0.39 increased odds of ICH, respectively (Table 3).

Discussion

Our preliminary study showed that blood pressure, serum HO-1, serum total bilirubin, and direct bilirubin were raised in patients with ICH who did not take medicine for hypertension compared with those who did, and increased in ICH patients in comparison with the control group. To the best of our knowledge, this is the first report on the elevated serum HO-1 level in patients with ICH.

Extracellular heme derived from hemoglobin following hemorrhage or released from dying cells induces the expression of heme oxygenase-1 (HO-1, HSP-32) which metabolizes heme to carbon monox-

Table 2 Serum concentrations of HO-1, total bilirubin, direct bilirubin, and blood pressure in ICH patients on medication to control hypertension regularly, irregularly, or never

	Compliant (n = 10)	Noncompliant (n = 16)	None (n = 14)	Reference	*P*
HO-1 (μmol/L)	131.51 ± 23.18	188.79 ± 44.85	275.14 ± 73.25	80.0–150.0	<0.001
TBIL (μmol/L)	9.37 ± 5.23	10.69 ± 4.86	10.31 ± 4.8	5.00–21.00	0.801
DBIL (μmol/L)	2.52 ± 1.41	2.83 ± 1.37	3.24 ± 1.61	0.00–7.00	0.485
SBP (mmHg)	143.5 ± 0.55	159.88 ± 19.53	167.36 ± 26.59	<140	0.046
DBP (mmHg)	92 ± 8.69	86.37 ± 9.47	94.85 ± 7.51	<80	0.033

HO-1, heme oxygenase-1; TBIL, total bilirubin; DBIL, direct bilirubin; SBP, systolic blood pressure; DBP, diastolic blood pressure.

After Kolmogorov–Smirnov test, differences between groups were tested with 1-way analysis of variance followed by the post hoc Bonferroni test.

Table 3 Multivariate regression studies on the risk factors of intracerebral hemorrhage

	OR	*P*
No compliance to medication	88.2	0.001
HO-1	0.96	0.003
SBP	0.93	0.004
Dbil	0.39	0.004

HO-1, heme oxygenase-1; SBP, systolic blood pressure; DBIL, direct bilirubin.

During multivariate regression, adjustment of age, gender, smoking, diabetes, and dyslipidemia ratio was performed; patients with hypertension who never take medication for hypertension had the highest odd of ICH in comparison with those who are on medication.

ide, iron and biliverdin. Biliverdin and its product bilirubin are powerful antioxidants for HO-1. The expression of the HO-1 is upregulated following ICH,[7] immunohistochemistry study showed that HO-1 proteins were highly detectable in the peri-ICH region predominantly in microglia/macrophages and endothelial cells after ICH.[9] The accumulated HO-1+ microglia/macrophages at the hemorrhagic lesion were detected as early as 6 hours after trauma and were still pronounced after 6 months. The upregulated HO-1 reached its peak at days 3 and 7 after ICH in Sprague-Dawley rats.[10]

For clinical study, HO-1 concentration in cerebral spinal fluid from 48 infants and children after traumatic brain injury increased in comparison with 7 control patients.[11,12] HO-1, bilirubin, and peroxidized lipid content were significantly higher in cerebral spinal fluid from subarachnoid hemorrhage patients with vasospasm, compared with patients with nonvasospasm, and correlated with occurrence of vasospasm.[12]

The role of HO-1 in ICH is controversial. HO-1 is thought to serve a protective antioxidant function, and upregulation of HO-1 has been demonstrated in experimental models of neurodegeneration, subarachnoid hemorrhage, cerebral ischemia, and traumatic brain injury. The early upregulation of HO-1 may be protective against oxidative stress, and overexpression in the late stages may lead to its dysfunction and be toxic.[10] Increased HO-1 concentration was described to be associated with the severity of injury and unfavorable neurologic outcome.[11] Even HO activity protects astrocytes from heme-mediated injury, but paradoxically increases neuronal injury.[13] However, in our study, it is hard to tell the protective or harmful effects of serum HO-1 to our patients.

A meta-analysis study showed that a negative relationship between bilirubin levels and severity of atherosclerosis.[11] The serum levels of direct bilirubin and total bilirubin were increased after acute ischemic stroke, which linked to the severity of stroke.[14] Total bilirubin levels were demonstrated to be decreased in carotid intima-media thickness, cardiovascular disease, stroke, and peripheral arterial disease. Multivariate logistic regression analysis revealed that higher total bilirubin was associated with a lower risk of silent cerebral infarction.[15] However, the bilirubin level in our ICH patients was higher than in control patients, and the discrepancy between our studies with above statement could be due to the bias caused by a limited number of our patients.

The hypertension especially poorly controlled hypertension due to poor compliance leads to a serious outcome, such as intracerebral hemorrhage.[16] Our results confirm that hypertension is a risk factor for ICH, and implied the importance of reduction of hypertension, adequate blood pressure monitoring, as well as encouraging compliance, in patients with established hypertension.

The limitations of this study include small number of ICH patients, and many confounding factors such as diabetes mellitus and hypertension in the control group that could bias the study results.

Overall, our preliminary study showed serum HO-1 level was higher in patients with ICH than without, and the blood pressure was a risk factor for ICH. Further investigation in multiple medical centers with large number of cohorts is warranted to verify our results.

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