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Elevated serum ubiquitin C-terminal hydrolase-L1 levels in patients with carbon monoxide poisoning

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ABSTRACT

Objective: Ubiquitin C-terminal hydrolase-L1 (UCH-L1) has been established as a reliable and potential biomarker of neuronal damage after acute neurologic insults, such as ischemic stroke, subarachnoid hemorrhage, and traumatic brain injury. However, the effect of serum UCH-L1 levels has not been investigated in carbon monoxide (CO)-poisoned patients. The aim of the present study was to evaluate whether serum UCH-L1 levels are a reliable marker of brain damage and the association of UCH-L1 with outcome.

Design and methods: This case–control study enrolled 46 CO-poisoned subjects and 30 controls. Using an enzyme-linked immunosorbent assay (ELISA) kit, we studied the temporal profile of serum UCH-L1 levels at 6, 12, 24 and 48 h after acute CO poisoning. Poisoning severity was assessed using the Glasgow Coma Scale (GCS) score. Long-term outcome was assessed using the Glasgow Outcome Scale (GOS) at 6 months after poisoning.

Results: Compared with controls, CO-poisoned patients had significantly elevated serum levels of UCH-L1 at each time point after poisoning. There were significantly higher levels of UCH-L1 in CO-poisoned patients with a lower GCS score as well as in those with a poor 6-month outcome dichotomized GOS.

Conclusions: Serum levels of UCH-L1 appear to have potential clinical utility in providing valuable information about poisoning severity and outcome after CO poisoning.

Introduction

Ubiquitin C-terminal hydrolase-L1 (UCH-L1), also known as neuronal-specific protein gene product (PGP 9.5, EC 3.4.19.12), is a highly brain-specific and abundant protein present in neurons. UCH-L1 is found in almost all neuronal tissue and approximates between 1 and 5% of total soluble brain protein [1]. It has been suggested that UCH-L1 plays a critical role in the removal of excessive, oxidized, or misfolded proteins during both normal and neuropathological conditions. Several studies have indicated that alterations in its activity are associated with neurodegenerative diseases, such as Parkinson's, Huntington's and Alzheimer's disease [2,3]. In addition, UCH-L1 may be a marker of neuronal loss after aneurysmal subarachnoid hemorrhage [4] as well as a marker of abnormal blood–brain barrier function after severe traumatic brain injury (TBI) [5]. A recently published study by Douglas-Escobar and colleagues demonstrated increases in serum UCH-L1 concentrations in neonates after moderate-to-severe hypoxic ischemic encephalopathy [6].

Carbon monoxide (CO) is the main cause of poisoning-related death and morbidity in developed countries and may be responsible for more than half of the fatal poisonings reported in many countries [7]. CO binds to hemoglobin 210 times more strongly than oxygen and impairs the transport and delivery of oxygen to tissues, often resulting in global hypoxic-ischemic brain damage [8]. CO exposure also causes inflammation through multiple pathways that are independent of those involved in hypoxia, leading to neurologic injury [9]. To the best of our knowledge, UCH-L1 levels in serum have not been previously measured after CO poisoning. We hypothesized that the serum UCH-L1 level is a novel marker of neuronal damage in patients suffering from CO poisoning. Therefore, the aim of this study was (i) to assess whether UCH-L1 was elevated in serum from CO-poisoned patients compared to control subjects, and (ii) to evaluate if UCH-L1 levels were associated with indicators of poisoning severity and outcome.

Materials and methods

Patients

This prospective controlled cohort study was conducted between June 1, 2010 and June 1, 2012 at the First Hospital of Jilin University. Adult patients with CO poisoning who presented to the emergency

Abbreviations: CO, carbon monoxide; COHb, carboxyhemoglobin; ED, emergency department; ELISA, enzyme-linked immunosorbent assay; GCS, Glasgow Coma Scale; GOS, Glasgow Outcome Scale; HBO, hyperbaric oxygen; HBOT, HBO therapy; NBO, normobaric oxygen; SEM, standard error of the mean; TBI, traumatic brain injury; UCH-L1, ubiquitin C-terminal hydrolase-L1.

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department (ED) within 6 h of poisoning, were enrolled in the study. The diagnosis of CO intoxication was established according to patient history, clinical characteristics, and blood carboxyhemoglobin (COHb) level. Exclusion criteria were (1) patients younger than 18 years of age; (2) mixed poisoning; (3) previous diagnosis of neuropsychiatric disease; (4) pregnancy; (5) cardiac arrest at the time of arrival in the ED; and (6) refusal to participate in the study. Control subjects were healthy volunteers who had a normal mental status at the time of enrollment and had no evidence of chronic CO exposure or hemodynamic instability. The study protocol was approved by the Medical Ethics Committee of the First Affiliated Hospital of Jilin University. Written informed consent was obtained from enrolled patients or in the case of unconscious patients, from relatives.

Data collection and processing

Patient demographics, cause of exposure, length of time exposed to CO exposure, symptomatology at presentation, systemic and neurological examination findings, Glasgow Coma Scale (GCS) score, serum COHb level, other laboratory results, and the modes of treatment were recorded. Poisoning severity was classified as mild (GCS score 13–15), moderate (GCS score 9–12), or severe (GCS score ≤8).

All patients received oxygen by open face mask during observation in the emergency department. Patients were treated with normobaric oxygen (NBO) or hyperbaric oxygen (HBO). The patients were accepted for HBO therapy (HBOT) according to the criteria of having transient or prolonged unconsciousness, abnormal neurologic findings upon physical examination, evidence of cardiac ischemia, or a COHb level >25%. Patients who were eligible for HBOT received treatment immediately after stabilization, initially compressed to 3.0 absolute atmospheres (abs atm) for 50 min, followed by 70 min at 2.8 atm abs and once a day for 5 sessions thereafter.

The Glasgow Outcome Scale (GOS) was assessed at 6 months after poisoning by direct patient contact or by telephone interview with the patient and/or a family member. The GOS score was determined using the standard neurologic parameters previously defined [10]. For the purpose of our analysis, outcome was classified into two groups; a poor outcome group (POG = GOS ≤ 3) and a good outcome group (GOG = GOS > 3).

Blood samples and measurement of serum UCH-L1

Blood samples were taken from the brachial vein of CO-poisoned patients at 6, 12, 24 and 48 h following poisoning. Approximately 5 ml of blood was collected from each subject at each sample point (within 60 min of the expected time point). Samples were centrifuged for 10 min at 5000 rpm and immediately frozen and stored at −70 °C until the time of analysis. Serum UCH-L1 levels were measured by a commercial enzyme-linked immunosorbent assay (ELISA) kit (Wuhan, China). Operation steps were performed according to the manufacturer's protocol. All samples were measured in duplicate. The intra-assay variability was 6% for UCH-L1 and the inter-assay variability was 9%. Optical densities were determined with a microplate reader set to a wavelength of 450 nm.

Statistical analysis

For statistical analysis, UCH-L1 levels were treated as continuous data, measured in μg/L and expressed as mean ± standard error of the mean (SEM). Data normality was assessed with the Kolmogorov–Smirnov test. Student's *t*-test and Mann–Whitney *U*-test were used for comparison of normally distributed and non-parametric data, respectively, between groups. The changes of the variables were tested using the Paired *t* test. Pearson correlation coefficient was used to correlate variables in the groups studied. A p value of <0.05 was accepted as statistically significant. The analysis of the data was performed using the SPSS version 17.0 (IBM, Inc., NY, USA).

Results

A total of 76 subjects were enrolled in the study and had serum samples obtained for analysis, including 46 CO-poisoned patients and 30 controls. Of the 46 CO-poisoned patients, 10 had severe poisoning (GCS score ≤8), 15 had moderate poisoning (GCS score 9–12), and 21 had mild poisoning (GCS score 13–15). The mean age of CO-poisoned patients was 38.4 ± 17.6 years with 45.7% being male, while the mean age of control participants was 42.3 ± 19.5 years with 56.7% being male. Although these weren't strictly age-matched, there was no difference in age or gender between CO-poisoned patients and controls. The main characteristics of the subjects are shown in Table 1.

The UCH-L1 levels were readily detectible at the earliest time point (i.e., within 6 h of estimated poisoning) as measured in serum. Forty-two (91.3%) poisoned patients had elevated UCH-L1 levels. Serum UCH-L1 concentrations at 6, 12, 24, and 48 h after poisoning were 9.14 ± 0.63, 8.29 ± 0.60, 6.95 ± 0.47, and 4.56 ± 0.35 μg/L, respectively. The CO-poisoned patients had significantly elevated serum levels of UCH-L1 after poisoning at each time point compared with controls (Table 2, Fig. 1).

The UCH-L1 levels were also compared against poisoning severity by analyzing GCS scores. The GCS score upon admission was used for this analysis. Patients with a GCS score of ≤8 (generally reflecting more severe poisoning) indeed had higher serum UCH-L1 levels than patients with GCS scores of 9 to 12 and GCS scores of 13 to 15 ($p < 0.001$, Table 2).

We next explored correlations between serum UCH-L1 levels at 6 h after poisoning and clinical parameters of patients (age, length of exposure to CO, COHb levels, GCS score on admission). We found that serum UCH-L1 levels correlated negatively with GCS score ($r = 0.90$, $p < 0.001$) (Fig. 2) where higher serum UCH-L1 levels

Table 1
Baseline demographic and clinical characteristics of the study population.

Variables	Control group (n = 30)	CO poisoning group (n = 46)
Age, mean ± SD (y)	42.3 ± 19.5	38.4 ± 17.6
Sex (M/F)	17/13	21/25
Sources of CO, n (%)		
Stoves (coal or wood)		29 (63.0%)
Water heaters		10 (21.7%)
Fires		7 (15.3%)
Length of exposure to CO, mean ± SD (h)		6.3 ± 2.2
Initial symptoms, n (%)		
Headache		38 (82.6%)
Dizziness		31 (67.4%)
Nausea		34 (74.0%)
Weakness		27 (58.7%)
Dyspnea		10 (21.7%)
Blurred vision		8 (17.4%)
Palpitations		13 (28.3%)
Confusion		5 (10.9%)
Syncope		8 (17.4%)
Ataxia		3 (6.5%)
Loss of consciousness		4 (8.7%)
COHb level, mean ± SD (%)		30.1 ± 6.8
ECG findings, n (%)		
Absent		27 (58.7%)
Sinus tachycardia		14 (30.4%)
ST changes		5 (10.9%)
GCS score on admission, mean ± SD		11.3 ± 3.7
Treatment applied, n (%)		
Normobaric oxygen therapy (NBOT)		26 (56.5%)
Hyperbaric oxygen therapy (HBOT)		20 (43.5%)