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Original Research Article

Overexpression of ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) in serum of children after thermal injury

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ABSTRACT

Purpose: The study aims to determinate concentrations of ubiquitin C-terminal hydrolase 1 (UCHL1), which hydrolyzes amino acids from ubiquitin and cleave di-ubiquitins, in serum of children after thermal injury.

Patients/methods: 42 children scalded by hot water, managed at the Department of Pediatric Surgery, with burns in 4–20% TBSA were included into the study (age 9 months up to 14 years, mean age 2.5 ± 1 years). Blood plasma UCHL1 concentration was assessed in 2–6 h, 12–16 h, 3d, 5d, and 7d after injury using surface plasmon resonance imaging biosensor. 18 healthy subjects admitted for planned surgeries served as controls.

Results: The UCHL1 concentration in the blood plasma of patients with thermal injuries reached its peak 12–16 h after thermal injury and slowly decreased over time, and still did not reach the normal range on the 7th day after thermal injury. Mean concentrations of UCHL1 after thermal injury were above the range measured in controls (0.12 ng/ml): 2–6 h after injury – 5.59 ng/dl, 12–16 h after injury – 9.16 ng/dl, 3 days after injury – 6.94 ng/dl, 5 days after 5.41 ng/dl, 7 days after injury – 4.09 ng/dl.

Conclusions: We observed sudden increase in the concentration of UCHL1 2–16 h after thermal injury with the slow decrease in the UCHL1 concentration over the time. UCHL1 concentration was proportional to the severity of the burn. Further studies are needed to determine the mechanisms by which UCHL1 contributes to metabolic response following thermal injury.

1. Introduction

Ubiquitin-mediated protein degradation plays a crucial role in various cellular processes, including signal transduction, cell differentiation, and stress response. Ubiquitin C-terminal hydrolase 1 (UCHL1) is a unique deubiquitinating enzyme that has both hydrolase and ligase activities. UCHL1 is expressed predominantly in the brain and neuroendocrine systems, and accounts for 1–2% of total brain soluble proteins [1]. It can remove ubiquitin from poly-ubiquitin chains, and also add ubiquitin to already ubiquitinated proteins [2–4]. Since inhibition of UCHL1 results in a 50% reduction of free ubiquitin in vitro, UCHL1 was suggested to be critically important for maintaining free ubiquitin levels and for the proper function of the ubiquitin-proteasome system [5,6]. UCHL1 effectively hydrolazes amino acids from ubiquitin and cleave di-ubiquitins. It also acts as a free ubiquitin stabilizer, providing ready-to-use ubiquitin for various cellular events. Neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, are closely related do dysregulation of UCHL1 [1,2]. UCHL1 is detected in cortical Lewy bodies and neurofibrillary tangles in patients with amyotrophic lateral sclerosis, Alzheimer's disease, and frontotemporal dementia [2,7,8]. Reduced UCHL1 levels are reported in amyotrophic lateral sclerosis and Alzheimer's disease patients [2,9]. In the absence of UCHL1 function, corticospinal motor neurons are prone to cellular degeneration, and undergo early and progressive cytoarchitectural defects, especially observed with disintegration of apical dendrites [2].

Aberrant expression of UCHL1 also contributes to cancer. Still its biological function in different types of cancer is controversial, studies on the subject found that UCHL1 is performing as an oncogene or a tumor suppressor [10]. Yu et al. recognized UCHL1 as

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a tumor suppressor involved in tumorigenesis of hepatocellular carcinoma and other digestive cancers, on the other hand UCHL1 is also considered as an oncogene in cancers including non-small lung cancer, lymphoma, prostate cancer, colorectal cancer, melanoma and osteosarcoma [10–16]. Some authors even postulate that UCHL1 could be a candidate biomarker and a therapeutic target for gastric cancer metastasis [10]. Moreover, latest studies show that pharmacological inhibition of UCHL1 blocks hepatic stellate cells proliferation and when administered in vivo acts in a therapeutic way to block progression of established fibrosis despite continued liver injury [17]. Despite many functions identified so far, there is no data available on the literature, on the expression of UCHL1 after different types of injury. Therefore we wanted to determinate concentrations of UCHL1 in serum of children after thermal injury.

2. Patients and methods

2.1. Patients

42 children scalded by hot water who were managed at the Department of Pediatric Surgery between 2013 and 2014, after primarily presenting with burns in 4–20% TBSA were included into the study (age 9 months up to 14 years, mean age 2.5 ± 1 years). There were 15 girls and 27 boys. Patients were divided into three groups depending on the severity of the burn according to American Burns Association: children with minor burns $n = 12$ (<5% TBSA burn, <2% full thickness burn), patients with moderate burns $n = 19$ (5–10% TBSA burn, 2–5% full-thickness burn), and patients with severe burns $n = 11$ (>10% TBSA burn, >5% full-thickness burn).

18 healthy, age matched subjects, subjects admitted for planned surgeries served as controls. Exclusion criteria were: hospital admission later than 6 h after thermal injury, severe preexisting infections, immunological or cardiovascular diseases that required long-term medication. All parents of our patients, gave written informed consent for both clinical and biochemical follow-up. The study had the Ethics Committee of University of Bialystok approval R-I-002/19/2011.

2.2. Methods

Venous blood samples (1–2 ml) were drawn 2–6 h (on the admission), and 12–16 h after the thermal injury, and on the subsequent days 3.5 and 7. Blood samples were collected in EDTA tubes, plasma prepared according to the standard protocols and stored at −80 °C. After all blood samples were collected and patient data recorded, the UCHL1 concentration was assessed using surface plasmon resonance imaging by the investigators blinded to the other data [18].

Blood plasma UCHL1 concentration was assessed in 2–6 h, 12–16 h, 3d, 5d, and 7d after injury using surface plasmon resonance imaging biosensor.

2.3. Procedure of UCHL1 determination

The ubiquitin carboxy-terminal hydrolase L1 (human recombinant UCH-L1, R&D System. Inc.) concentration was determined using the surface plasmon resonance imaging (SPRI) biosensor. The exact description of the methodology of measurements and biosensor design is set out in the previous paper [18]. Gold chips were manufactured as described in other papers [19,20]. The gold surface of the chip was covered with photopolymer and hydrophobic paint.

Chips were rinsed with ethanol and water and dried under a stream of nitrogen. They were then immersed in 20 mM of cysteamine ethanolic solutions for 2 h and after rinsing with ethanol and water dried again under a stream of nitrogen [20]. The rabbit monoclonal IgG_{2A} antibody specific for human UCHL (R&D System, Inc.) were immobilized on the thiol monolayer under the suitable conditions. The antibody solution in a PBS buffer was activated with NHS (250 mM) and EDC (250 mM). Activation of the antibody was done by adding the mixture of NHS and EDC (1:1) in a carbonate buffer solution (pH 8.5) into the antibody solution and with vigorous stirring for 5 min at the room temperature. 3 μl of this solution was placed on the active places with the amine-modified surface, and incubated at 37 °C for 1 h [21].

After this time the biosensor were rinsed with water. Next, serum samples (10× diluted) were placed directly on the prepared biosensor. The volume of the sample applied on each measuring field was 3 μl. Time of the interaction with antibody was max 10 min. The biosensor was washed with water and HBS-ES buffer solution pH = 7.4 (0.01 M 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid, 0.15 M sodium chloride, 0.005% Tween 20, 3 mM EDTA, BIOMED, Lublin, Poland) to remove unbound molecules from the surface.

SPRI measurements for protein array were performed as described in the previous papers and schematic diagram apparatus is given in the paper [22]. As controls of the level of non-specific binding some of the places on the biosensor covered with buffer were used.

The SPRI signal was measured at a fixed SPR angle on the basis of registered images. The first image after immobilization of the antibody was taken. Then, the second image after interaction with UCH-L1 was taken. The SPRI signal was obtained by subtraction of the signal after and before interaction with a biomolecule, for each spot separately. The contrast values obtained for all pixels across a particular sample single spot were integrated. Then, the SPRI signal was integrated over the spot area. NIH Image J version 1.32 software was used to evaluate the SPRI images in 2D form and to convert of numerical signal to a quantitative signal (A.U.).

2.4. Statistics

The Mann–Whitney U test and the Kruskal–Wallis H test with Dunn's post hoc correction to control for multiple testing were used to compare differences between groups. Statistical analyses were calculated with the STATISTICA PL release 10.0 program. A two-tailed $p < 0.05$ was considered significant.

3. Results

The UCHL1 concentration in the blood plasma of patients with thermal injuries was above the range of concentrations measured in controls. The UCHL1 concentration reached its peak 12–16 h after thermal injury and slowly decreased over time, and still did not reach the normal range on the 7th day after thermal injury ($p < 0.05$) (Fig. 1). Mean concentrations of UCHL1 after thermal injury were above the range measured in controls (0.12 ng/ml): 2–6 h after injury – 5.59 ng/dl, 12–16 h after injury – 9.16 ng/dl, 3 days after injury – 6.94 ng/dl, 5 days after injury 5.41 ng/dl, 7 days after injury – 4.09 ng/dl. UCHL1 concentration was proportional to the severity of the burn. The difference was statistically significant ($p < 0.05$) (Fig. 2). We did not note any severe complications in our burned patients. All patients were discharged home on the final stage of wound healing, in good general state and had further treatment in the surgical ambulatory.

4. Discussion

Burn injury initiates a dramatic systemic response in humans. In the view of prospecting for progress in therapy, there is a developing need for the generation and characterization of novel