



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.

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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 hydrolyzes 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 to 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 Białystok 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 $\times$ 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

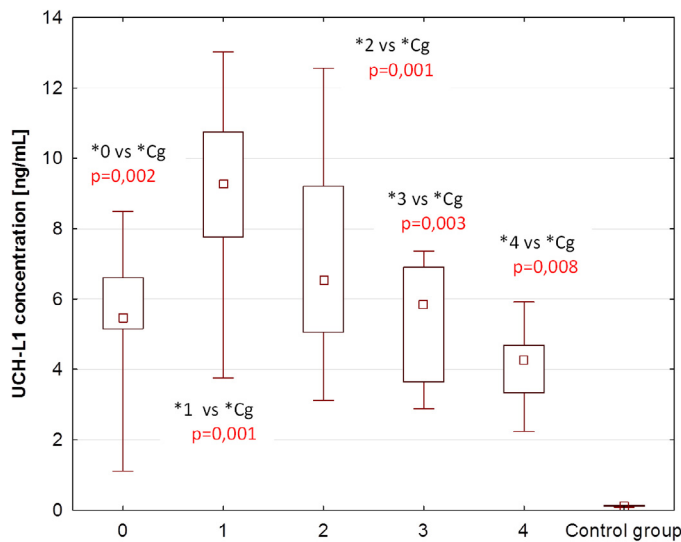


Fig. 1. UCH-L1 concentration in the plasma of children, 2–6 h (0, $n = 42$), 12–16 h (1, $n = 42$), 3 days (2, $n = 37$), 5 days (3, $n = 35$), 7 days (4, $n = 27$) after thermal injury and in control group (Cg).

tools that mimic human pathology at a cellular level. Ubiquitin-mediated protein degradation plays a crucial role in various cell processes, including signal transduction, cell differentiation, and stress response [23]. The ubiquitin system is an important post-translational regulator of many cellular processes including protein turnover and cell signaling. Indices of calcium-activated proteolysis [2], ubiquitin-proteasome activity [24], and apoptosis [25], have all been implicated in burn-induced muscle atrophy in rats. On the other hand, deubiquitination system can prevent protein degradation, by reversing the ubiquitination process via the disassembly of the poly-ubiquitin chain, recycling active ubiquitin by the removal of ubiquitin from its covalently linked protein, and generating monomeric ubiquitin from its precursor fusion protein [23]. The net ubiquitination status of any protein is regulated both through the processes of attachment and detachment with alterations of either event leading to changes in the half-life or biochemical function of targeted proteins [26,27]. UCHL1 is

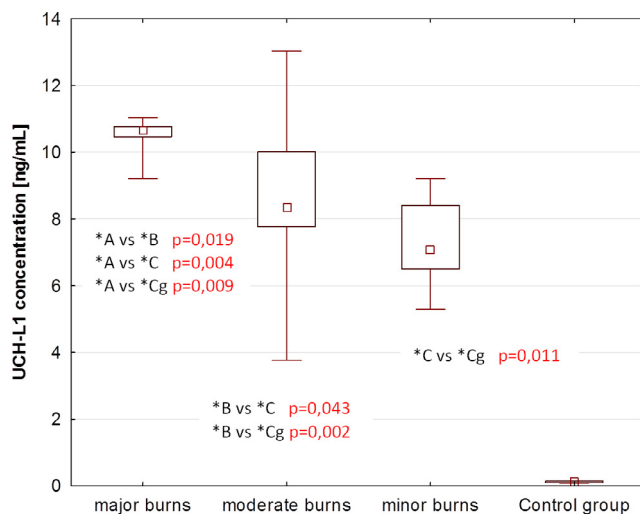
able to function as both a ubiquitin ligase and deubiquitinase. UCHL1 can also interact and stabilize cell proteins such as β catenin, and activate kinases such as CDK4 without the requirement for its enzymatic activity [17,28,29]. The function of UCHL1 probably varies in a cell and environmental specific manner. UCHL1 exerts its effects by influencing levels of free proteins such as cellular ubiquitin, glutathione and by regulation of the cell cycle [12,17,30]. UCHL1 was first identified as an abundant protein found in the brain and testes [17], however its expression is not limited to the neuronal compartment [6]. UCHL1 is also highly expressed in carcinomas of various tissue origins, including those from brain, lung, breast, kidney, colon, prostate, pancreas, and mesenchymal tissues [23]. Loss-of-function studies and an inhibitor for UCHL1 confirmed the importance of UCHL1 for cancer therapy. Moreover, expression levels of UCHL1 in breast and lung cancers were found to be associated with those of HIF-1 α and poor prognosis in cancer patients [31]. Previous studies shown that UCHL1 is involved in tumor metastases [12,31–33]. We observed abrupt rise the UCHL1 concentration in the blood plasma of patients after thermal injuries, which reached its peak 12–16 h after the burn and slowly decreased over time. UCHL1 concentration was proportional to the severity of the burn, so we believe that its expression reflects metabolic response to stress caused by thermal injury.

There are observations, that UCHL1 can also modulate vascular remodeling [23]. UCHL1 attenuated tumor necrosis factor- α -induced (TNF- α) nuclear factor κ B (NF- κ B) activity and increased eNOS expression, which may directly attenuate arteriosclerosis leading to the reduction of ischemic vascular disease, and nuclear factor κ B (NF- κ B) remains the most important link between the ubiquitin-proteasome system and inflammation and by this with the response to the thermal injury [23,34].

The unexpected success of the proteasome inhibitor bortezomib in the treatment of multiple myeloma focuses the attention on the role of the ubiquitin-proteasome system and possible novel targets in therapy. Also treatment of cardiovascular cells with proteasome inhibitors results in strong antiproliferative, anti-inflammatory, and proapoptotic effects [23,35]. In animal model, UCHL1 deletion ameliorates glomerular injury in mice with focal segmental glomerulosclerosis [30]. The observation that in lymphoma cells, UCHL1 knockdown decreases cellular growth and viability, may in the future contribute to evolvement of novel therapies [12,17,36]. A possible role for overexpression of UCHL1 after thermal injury is still not known. We believe that, there is need for a wider examination of the role of the ubiquitin system and its role in the metabolic response after burns.

In the literature, the information about UCHL1 and its relation to the inflammation process or tissue injury, pain or healing process is scarce. It has been demonstrated that the expression of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is increased in injured podocytes in a number of types of glomerulonephritis. It is postulated that the activation of NF- κ B and upregulation of UCH-L1 in podocytes may be vital in podocyte injury associated with immune complex-mediated glomerulonephritis [37]. Furthermore a functional NF- κ B response element was identified in the UCH-L1 promoter region. Expression of NF- κ B suppressed UCH-L1 gene transcription. In the RelA knockout system where NF- κ B activity is ablated, UCH-L1 expression was significantly increased. Activation of NF- κ B signaling by the inflammatory stimulator lipopolysaccharide and TNF α resulted in a decrease of UCH-L1 gene expression by inhibiting its transcription. There is possibility that inflammation might compromise neuronal functions via the interaction of NF- κ B and UCH-L1 [38].

Some authors suggest that, endothelial UCHL1 might also influence intraocular neovascularisation [39]. UCHL1 also works well as real-time indicator of pancreatic beta cell damage



*A- major burns, B- moderate burns, C-minor burns, Cg-control group

Fig. 2. UCH-L1 concentration 12–16 h after thermal injury in the plasma of children with major, moderate and minor burns (burn area and burn depth).

in vitro. Moreover whole-body dynamic planar imaging of (99 m)-Technetium-labeled UCHL1 indicated a rapid UCHL1 uptake in the liver and spleen, followed by urinary excretion of mainly proteolytic UCHL1 fragments [40]. To the best of our knowledge, our study is the first information about the overexpression of UCHL1 in thermal injury, further studies are needed to determine biological meaning of such phenomenon.

5. 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.

Conflict of interests

The authors declare no conflict of interests.

Financial disclosure

The authors have no funding to disclose.

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