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Matrix metalloproteinase-2 and its correlation with basal membrane components laminin-5 and collagen type IV in paediatric burn patients measured with Surface Plasmon Resonance Imaging (SPRI) biosensors

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ABSTRACT

The purpose of this study was the determination of matrix metalloproteinase-2 and its correlation with basal membrane components laminin-5 and collagen type IV in the blood plasma of burn patients measured with Surface Plasmon Resonance Imaging (SPRI) biosensors.
Material and methods: 31 children scalded by hot water who were managed at the Department of Paediatric Surgery between 2014–2015, 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 10 girls and 21 boys. Venous blood samples were drawn 2–6 h, and 12–16 h after the thermal injury, and on the subsequent days 3, 5 and 7. The matrix metalloproteinase-2, collagen type IV and laminin-5 concentrations were assessed using Surface Plasmon Resonance Imaging by the investigators blinded to the other data.
Results: The MMP-2, laminin-5 and collagen type IV concentrations in the blood plasma of patients with burns, were highest 12–16 h after thermal injury, the difference was statistically significant. The MMP-2, laminin-5 and collagen type IV concentrations measured 3 days, 5 days and 7 days after the thermal injury, slowly decreased over time, and on the 7th day reached the normal range, when compared with the concentration measured in controls.
Conclusion: Current work is the first follow-up study regarding MMP-2 in burns. MMP-2, laminin-5 and collagen type IV levels were elevated early after burn injury in the plasma of studied patients, and were highest 12–16 h after the injury. MMP-2, laminin-5 and collagen type IV levels were not proportional to the severity of the burn. We believe in the possibility that the gradual decrease of MMP-2, collagen type IV and laminin-5 concentrations could be connected with the process of healing, but to prove it, more investigation is needed in this area. The SPR imaging biosensor is a good diagnostic tool for determination of MMP-2, laminin-5 and collagen type IV in blood plasma of patients with burns.

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1. Introduction

Thermal injury is an extremely common, but very complex, clinical challenge, and still remains a leading cause of childhood death [1]. Severe burns are associated with systemic inflammatory response syndrome (SIRS) which in the early post-burn period alter proinflammatory and anti-inflammatory cytokines [2]. One of the post-burn changes is upregulation of metalloproteinases (MMPs), a major group of zinc-dependent endopeptidases, that contribute to remodelling of the extracellular matrix (ECM) by degrading proteins and polysaccharides including basement membrane components (i.e. collagen type IV and laminin) [1,3]. Wound extracellular matrix is a regulator of cell adhesion, migration, proliferation and differentiation during cutaneous repair. The structure of normal wound ECM is determined by a dynamic balance among matrix synthesis, deposition, and degradation [4].

The matrix metalloproteinases are produced by a number of cell types involved in wound repair, including fibroblasts, macrophages, endothelial cells, and keratinocytes [2,5]. MMPs function is not only reduced to degradation of the ECM, they have a key role in the wound healing process [6]. MMPs also modulate the inflammatory response, due to degradation of basal membrane, they are responsible for migration and extravasation of leukocytes [7]. MMPs are also considered as signalling proteases — tissue inhibitors of metalloproteinases (TIMPs) can activate granulocytes and are able to protect inflammatory cells from apoptosis [2,8].

In animal models, Schaffer et al. reported the presence of mRNA for MMP-2 and MMP-9 during the healing of wounds caused by lasers, excisions or burns [9]. MMP-2 degrades not only extracellular matrix proteins (collagen type I, III, IV, V, VII, X, XI, gelatin, elastin, ecorin, aggrecan, fibronectin, laminin, tenascin and vitronectin) but also several nonmatrix proteins such as interleukin 1 beta, protransforming growth factor beta (pro-TGFβ), pro-tumor necrosis factor (pro-TNF) and others [6]. Under physiological conditions, MMP-2 is involved in remodelling of the vasculature, tissue repair, angiogenesis, inflammation, tumor invasion, and atherosclerotic plaque rupture. Other researchers have also shown significant elevation of MMP-2 and MMP-9 mRNA during healing of burn wound, but still did not fully elucidate their association with thermal injury [1,10].

To determine the MMP-2 concentration in body fluids the immunohistochemistry (ELISA) method can be used [11]. An alternative method is Surface Plasmon Resonance Imaging (SPRI). So far several SPRI biosensors were used for clinical research to determinate e.g. lysosomal proteases, 20S proteasome, ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) and immunoproteasome [12–14]. SPRI is based on the dependence of light reflectivity on the number of molecules adsorbed on the surface. Surface plasmon resonance (SPR) is an optical detection process that occurs when a polarized light hits a prism covered by a thin (gold) metal layer. Under certain conditions (wavelength, polarization and incidence angle) free electrons at the surface of the biochip absorb incident light photons and convert them into surface plasmon waves [6]. A dip in reflectivity of the light is seen under these SPR conditions. Perturbations at the gold surface of the biochip, such as an interaction between probe molecules immobilized on the chip and captured target molecules, induce a modification of resonance conditions which are in turn seen as a change in reflectivity and which can be measured. This technique uses two types of very specific interactions: antibody-antigen or inhibitor-enzyme [6].

Therefore the purpose of our study was to investigate the relationship between MMP-2 and thermal injury severity, and its correlation with basal membrane components — laminin-5 and collagen type IV, using novel technique Surface Plasmon Resonance Imaging (SPRI).

2. Methods

2.1. Patients

We included into the study 31 consecutive paediatric patients, with peripheral burn wounds, caused by hot water, who were admitted to the Department of Paediatric Surgery between 2014–2015, because of burns in 4–20% TBSA (age 9 months up to 14 years, mean age 2,5+1 years) (Table 1). Among them were 10 girls and 21 boys. Our patients were divided into three groups depending on the severity of the burn according to American Burns Association: children with minor burns n=8 (<5% TBSA burn, <2% full thickness burn), patients with moderate burns n=13 (5–10% TBSA burn, 2–5% full-thickness burn), and patients with severe burns n=10 (>10% TBSA burn, >5% full-thickness burn). The control group represented 18 healthy, age matched subjects, admitted for planned herniotomy between 2014 and 2015 (for this type of surgery, children are admitted to the department after paediatric examination and after excluding infections or prolonged medication). Exclusion criteria were: admission to the hospital later than 6h after thermal injury, immunological or cardiovascular diseases that required long-term medication, and severe preexisting infections. 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. The patients received standard routine care according to accepted guidelines.

2.2. Materials

As a standard, Recombinant Matrix Metalloproteinase-2 (Sigma Steinheim, Germany), collagen type IV (Sigma, Steinheim, Germany) and recombinant human protein laminin-5 (Abcam, USA) was used. ARP 101 — selective MMP-2 inhibitor ((2R)-2-[([1,10-biphenyl]-4-ylsulfonyl)(1-methylethoxy)amino]-*N*-hydroxy-3-methyl-butanamide, M1/4 405.50kDa, Sigma Steinheim, Germany), purified monoclonal mouse anti-human collagen type IV (Tebubio, France), rabbit polyclonal antibody specific to human laminin-5 (Abcam, USA), 1-octadecanothiol (ODM), 2-amino-ethanethiol hydrochloride (cysteamine hydrochloride), *N*-ethyl-N0-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS) and 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt (HEPES) (all Aldrich, Steinheim, Germany), photopolymer ELPEMER SD 2054, and

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