ASSESSMENT OF GLUCURONOSYL EPIMERISATION OF DERMATAN SULFATE CHAINS IN THE COURSE OF BURNED WOUND HEALING

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Received for publication April 18, 2010

Abstract

The objective of this study was to assess the extent of glucuronosyl epimerisation of dermatan sulfate (DS) chains, isolated from the matrix of burned wound bed. Dermatan sulfates were isolated and purified from normal and injured skin of domestic pigs, on days 3, 5, 10, 15, and 21 after the thermal damage. The wounds were treated with Propol T, silver sulfadiazine (SSD), physiological salt solution, and vehicle of Propol T. The isolated DS samples were depolymerised with chondroitinase ABC and chondroitinase B. The assessment of the amount of unsaturated disaccharides, released during DS enzymatic digestion was performed. It was found that in the course of the tissue repair the glucuronosyl epimerisation pattern of DS chains derived from burned wounds was altered as compared with epimerisation of DS isolated from normal skin. Propol T, in contrary to routinely used SSD, exerts a beneficial effect on DS metabolism leading to the formation of iduronate residue number similar to that of normal skin. The obtained results demonstrate that Propol T modulates DS structure resulting in the accelerated repair of burned tissue.

Key words: swine, burns, Propol T, silver sulfadiazine, epimerisation, dermatan sulfate.

Wound repair is a dynamic interactive process comprising many precisely interrelated stages, that reflect the complex and coordinated body response to tissue damage resulting from the interaction of many cell types and different extracellular matrix constituents — including proteoglycans (PGs) and glycosaminoglycans (GAGs) (17, 19). The main GAG occurring in the burned wounds, regardless of the stage of repair process, is dermatan sulfate (DS) (20). DS is actively involved in many biological stages of healing process i.e. coagulation, inflammation, and remodelling (15, 25). Iduronate (IdoA) residues of DS, mediating these biological activities, are created by DS epimerases DS-epi1 and DS-epi2 (12, 15). The first one is responsible for the majority of epimerase activity in the skin. DS-epi1 is the main enzyme producing iduronate blocks in the course of DS biosynthesis (12). The mentioned process consists of the formation of the linkage region joining glycan to the core protein of DS proteoglycan (DSPG), the creation of repeating disaccharides containing D-glucuronic acid (GlcA) and N-acetylgalactosamine (GalNAc) units, followed by the epimerisation and sulfation of the formed polysaccharide chain (7). It was suggested that His-450 of DS epimerase acts as the initial basis abstracting the C5 proton from GlcA. The following fission of the glycosidic linkage by Tyr-261 creates an 4,5-unsaturated intermediate, which is reprotonated from the opposite side of the saccharide plane by His-205 with a simultaneous formation of the glycosidic bond (15). After the epimerisation, the sulfation takes place because only non sulfated glycan can be the substrate for the DS-epimerase (3). 4-O-sulfation occurring after conversion of GlcA into IdoA prevents back epimerisation and increases the extent of epimerisation (20). In addition, sulfation of hydroxyl groups may occur at the 6-position of the GalNAc and at the 2-position of the IdoA (and to lesser extent at the 2-position of the GlcA) (15, 20). The described modifications, which create structural DS variability, produce specific chain sequences responsible for binding to proteins, and the biological effects of particular interest in the field of wound healing (12). The extent of the DS epimerisation can be changed in the course of various physiological and pathological processes. Hence, the objective of this study was to assess the glucuronosyl epimerisation of DS, isolated from the burned wounds.
Material and Methods

Experimental animals. The study protocol was approved by the Ethics Committee of the Medical University of Silesia. Four, 16 weeks old, domestic pigs have been chosen as the experimental animals. The pig has been proved to be the useful animal for the evaluation of wound repair because of many similarities of pig and human skin (8, 24). The pig body weight at the start of the experiment was 35-40 kg. The pigs were housed according to G.L.P. standards of Polish Veterinary Law. Animals’ state was evaluated by regular control of behaviour, body weight, and temperature. Seventy-two burn wounds were inflicted according to Hoekstra et al. (8) standard model. The animals were divided into control and experimental groups, each containing two animals. Control pig burn wounds were treated with physiologic saline to observe the healing process occurring without treatment (one animal) or with Propol T (propolis preparation, APIMED, Poland) vehicle (another animal), twice a day, during the whole experiment. Burn wounds of the experimental animals were treated with Propol T (one animal) or Dermazin cream (1% silver sulfadiazine, SSD, Sandoz/Lek Polska) (another animal) - twice a day, from the 1st to the 21st d of the experiment. Biopsies, in three replications, were taken from normal skin (day 0) and after burn infliction from the wound bed, on post-burn days 3, 5, 10, 15, and 21.

Extraction of tissue GAGs. GAGs isolation was carried out according to Scott (18) and Van Amerongen et al. (23). Briefly, tissue samples, after homogenisation with acetone, were digested with papain to release GAG chains from PG core proteins. Peptides generated by papain action, as well as protein resistant to the enzyme were removed by precipitation with trichloroacetic acid. Subsequently, GAGs were dialysed, precipitated with ethanol, dissolved in potassium trichloroacetic acid. Subsequently, GAGs were dialysed, precipitated with ethanol, dissolved in potassium acetate, and reprecipitated. The total amounts of GAGs were quantified by a hexuronic acid assay (2).

Assay of tissue GAGs. Samples of isolated GAGs were submitted to electrophoresis on cellulose acetate, before and after the use of enzymes specifically eliminating particular GAG types, i.e. chondroitinase ABC (pH 6.0), chondroitinase ABC (pH 8.0), and chondroitinase B (pH 7.5) (Sigma Aldrich, Poland) (6).

Electrophoresis of GAGs. Electrophoretic fractionation of GAGs was performed as described previously (9) (Fig. 1).

Epimerisation assessment. The content of DS disaccharides including IdoA and GlcA was assessed by the measurement of the absorbance at 232 nm, demonstrated on the basis of the obtained unsaturated products of DS degradation with chondroitinase ABC and B, respectively (6, 10).

Statistical analysis. Statistical differences between groups were determined by analysis of variance (ANOVA), followed by Tukey’s post-hoc tests. P<0.05 was considered to indicate statistical significance.

Results

The electrophoretic analyses of tissue GAGs allowed to identify DS, DS HS/H, and HA (Fig. 1). The majority of GAGs was identified as DS. An increase in DS content during the healing process (days 0-15), particularly visible after Propol T treatment, was followed by the reduction in DS amount after the 15th d of the study. When SSD was applied, the DS content was growing, although to a lesser extent, until the end of the experiment. The NaCl and Propol T vehicle led to the slight elevation of DS content. The differences in the DS content between the first and the last day of the experiment were statistically significant (Table 1).

It was also found that in the course of the tissue repair, the glucuronosyl epimerisation pattern of DS chains derived from burned wounds was altered to an extent depending on the applied agent. A reduction in IdoA residues content during the healing process (days 0-15), after Propol T application was followed by the increase in IdoA amount after the 15th d of the study. Burns treatment with SSD led to the decrease in IdoA content till the 10th d of the experiment and to the subsequent elevation of the IdoA residues level. NaCl led to the moderate increase in IdoA amount. Application of the Propol T vehicle changed the IdoA content marginally. The differences in IdoA residues content between the first and the last day of the experiment were statistically significant. An increase in GlcA residues content during the healing process (days 0-15), particularly visible after Propol T treatment, was followed by the reduction in GlcA amount after the 15th d of the study. When SSD was applied, the GlcA content was growing until the 10th d of the experiment. In the next days the decrease in GlcA residues was observed. NaCl led to the reduction in GlcA content. After the treatment with Propol T vehicle, GlcA level changed moderately. The differences in GlcA residues content
between the first and the last day of the experiment were statistically significant. The results obtained are presented in Fig. 2.

**Discussion**

The well known sequence of biochemical changes of DS during the different phases of tissue remodelling (19) allowed us to apply the experimental model of cutaneous wound repair in which the evaluation of therapeutic efficacy of Propol T was performed through the qualitative and quantitative DS analysis. DS is the predominant GAG expressed in the normal skin and in the burned wound bed (21). DS chains play an active role in the healing process throughout the regulation of the cell adhesion, migration and proliferation (16). These processes are connected with DS ability to bind numerous growth factors and potentiate their biological activity (20). Epimerisation of GlcA into IdoA is the process of extreme importance for wound healing (21). IdoA residues provide chain a considerable conformational flexibility of GAG, due to the easy changing between chair and skew conformations (27). The conformational flexibility of IdoA residues in DS promotes apposition too, and subsequently functional interactions with a variety of proteins at cell surfaces and in the extracellular matrix (22). IdoA domains play a key role in specificity of binding site for GAG-binding proteins, being essential for the PGs binding to collagen fibrils and for DS binding to several growth factors (15, 21). In the present study we compared the influence of apitherapeutic agent Propol T and SSD on DS accumulation and epimerisation pattern. Propol T, well known for its antimicrobial, anti-inflammatory, and healing time-reducing properties (5) was used for the first time to treat the minor skin burns. SSD applied in the present study, as an agent of choice for the outpatient management of partial-thickness burns, may place patients at increased risk of some side effects (4).

We have found that Propol T stimulated in a greater degree DS accumulation in the burn wounds than SSD did. The obtained results are in agreement with those described by Simeon et al. (19).

They have found that rat skin wounds treated with complex (Gly-his-lys) – Cu²⁺ (GHK-Cu), the healing activator, led to an elevation in DS amount, particularly visible between the 18th and 22nd d of the experiment.

Table 1

<table>
<thead>
<tr>
<th>Days</th>
<th>Propol T</th>
<th>SSD</th>
<th>NaCl</th>
<th>Propol T vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.046 ± 0.0294</td>
<td>1.093 ± 0.0676</td>
<td>1.073 ± 0.0238</td>
<td>0.99 ± 0.0312</td>
</tr>
<tr>
<td>3</td>
<td>1.089 ± 0.0087</td>
<td>0.891 ± 0.0312</td>
<td>0.515 ± 0.034</td>
<td>0.566 ± 0.0295</td>
</tr>
<tr>
<td>5</td>
<td>2.154 ± 0.0658</td>
<td>1.537 ± 0.0243</td>
<td>1.129 ± 0.026</td>
<td>0.732 ± 0.0226</td>
</tr>
<tr>
<td>10</td>
<td>3.897 ± 0.0379</td>
<td>3.513 ± 0.0413</td>
<td>1.562 ± 0.0303</td>
<td>1.232 ± 0.0331</td>
</tr>
<tr>
<td>15</td>
<td>5.856 ± 0.0494</td>
<td>4.843 ± 0.036</td>
<td>2.287 ± 0.021</td>
<td>2.447 ± 0.035</td>
</tr>
<tr>
<td>21</td>
<td>4.995 ± 0.0413</td>
<td>5.098 ± 0.0236</td>
<td>2.324 ± 0.0345</td>
<td>2.984 ± 0.033</td>
</tr>
</tbody>
</table>

± - SD

Fig. 2. The dynamics of IdoA and GlcA content changes in normal skin samples (■) and burned ones treated with Propol T (▪), SSD (●), NaCl (□), and Propol T vehicle (○○).
We suggest that Propol T action resembles that of GHK-Cu, described as a growth factor for differentiated cells, a chemotactic agent for monocytes/macrophages and mast cells, supporting angiogenesis, as well as enhancing the expression of ECM macromolecules both in vitro and in vivo (19). We have also observed that in the course of burn healing, DS undergoes significant structural changes, depending on the applied agent. Propol T stimulated the reduction in IdoA residues content till the 15th d of the experiment, followed by the increase in IdoA amount up to the values resembling those of normal skin. The similar tendency of changes was found in the case of SSD application, although the alterations were expressed to a lesser extent. The pattern of DS epimerisation observed after Propol T implementation is in agreement with that described by Kuwaba et al. (11). They have found that 2,4-dinitrofluorobenzen (DNFB), applied in the mice skin healing model led to the elongation of the DS chains and reduction of the quantity of the IdoA2S-GALNAc4S units on day 15 of the experiment followed by the enhancement in IdoA residues (11). Similar tendency was noted with regard to Propol T effect on IdoA content. Propol T may be also responsible for inducing the production of TGF-β known for the ability to reduce IdoA formation (1), needed in controlling collagen fibrillogenesis during the tissue restoration (26). Our previous studies showed that Propol T accelerates regenerative and reconstructive processes, reduces healing time, and displays higher than SSD antimicrobial efficacy (13, 14). The present results demonstrate that Propol T modulates DS metabolism leading to the effective repair of the burned tissue.

Acknowledgments: This work was supported by grant from the Medical University of Silesia, Poland (NN-2-346/03).

References
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