Propolis modulates vitronectin, laminin, and heparan sulfate/heparin expression during experimental burn healing*

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Abstract: Objective: This study was aimed at assessing the dynamics of vitronectin (VN), laminin (LN), and heparan sulfate/heparin (HS/HP) content changes during experimental burn healing. Methods: VN, LN, and HS/HP were isolated and purified from normal and injured skin of domestic pigs, on the 3rd, 5th, 10th, 15th, and 21st days following thermal damage. The wounds were treated with apitherapeutic agent (propolis), silver sulfadiazine (SSD), physiological salt solution, and propolis vehicle. VN and LN were quantified using an immunoenzymatic assay and HS/HP was estimated by densitometric analysis. Results: Propolis treatment stimulated significant increases in VN, LN, and HS/HP contents during the initial phase of study, followed by a reduction in the estimated extracellular matrix molecules. Similar patterns, although less extreme, were observed after treatment with SSD. Conclusions: The beneficial effects of propolis on experimental wounds make it a potential apitherapeutic agent in topical burn management.

Key words: Apitherapeutic agent, Silver sulfadiazine, Laminin, Vitronectin, Heparan sulfate/heparin, Wound healing
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1 Introduction

Propolis possesses a plethora of biological and therapeutic mechanisms, such as immunomodulatory, antitumor, antiinflammatory, antioxidant, antibacterial, antiviral, antifungal, and antiparasitic actions and can accelerate wound healing and reepithelialization (McLennan et al., 2008; Sforcin and Bankova, 2011). Particular propolis compounds have been extensively analyzed; however, little experimental research of its effects on burn wound healing has been carried out (Sforcin and Bankova, 2011). Skin repair after injury proceeds via a finely-tuned pattern of integrated phases: hemostasis, inflammation, proliferation, and remodeling, which all involve a number of cellular and molecular processes (Tong et al., 2008; Goh et al., 2010; Liu et al., 2010; Groah et al., 2011). These include migration and proliferation of epidermal cells and keratinocytes, fibroblast adherence, and extracellular matrix (ECM) contraction (Chen et al., 2005; Goh et al., 2010; Kikkawa et al., 2010). These mechanisms require deposition not only of multidomain adhesive glycoproteins such as laminin (LN) and vitronectin (VN) but also of glycosaminoglycans (GAGs) such as heparan sulfate/heparin (HS/HP) (Schultz and Wysocki, 2009). LN is essential for cell-cell recognition, differentiation, cell survival, and force transmission (Voermans et al., 2008). This omnipresent glycoprotein of all basement membranes is composed of three distinct subunits (α-, β-, and
γ-chains), which oligomerize to form a cruciform or T-shaped heterotrimer (Voermans et al., 2008; Durbeej, 2010; Roediger et al., 2010). In vertebrates, five α, three β, and three γ chains, have been identified to create 18 different LN isoforms (Durbeej, 2010). Each LN chain contains specific domains capable of interacting with cellular receptors such as integrins and extracellular ligands including heparan sulfate proteoglycans (HSPGs) (Tzu and Marinkovich, 2008; Durbeej, 2010). Binding of LN to cell surface receptors facilitates its multimerization (Ragbow et al., 2006). Another multiadhesive glycoprotein, VN, present in plasma as a single-chain (75 kDa) or two-chain (10 and 65 kDa) form, undergoes a multimerization process in the ECM (Ekmekçi and Ekmekçi, 2006; Sano et al., 2007). Human VN, also known as a serum-spreading factor or complement S-protein, consists of four structurally different domains: somatomedin B (SMB), hemopexin 1 (N-glycosylated), hemopexin 2, and a connecting region linking the somatomedin and hemopexin 1 domains (Ekmekçi and Ekmekçi, 2006; Piccard et al., 2007). Due to the Arg-Gly-Asp (RGD) amino acid sequence occurring in the SMB domain, VN binds integrins and serves as a cell attachment site promoting cellular adhesion and spreading (Ekmekçi and Ekmekçi, 2006). VN and LN interact with HS/HP proteoglycans (Beauvais et al., 2009; Durbeej, 2010). HS/HP is attached by covalent linkage to the core protein, forming in vivo HSPG (Wegrowski et al., 2006). HS/HP is a linear polymer consisting of repeating disaccharide subunits composed of α(1→4) linked uronic acid (either D-glucuronic acid (GlcA) or L-iduronic acid (IdoA)) and D-glucosamine (Wang et al., 2010; Malavaki et al., 2011). HS is less sulfated with lower IdoA content compared with HP—the highest negatively charged GAG (Malavaki et al., 2011). HS/HP is recognized as a pivotal player in angiogenesis, cell growth, migration, and differentiation (Wegrowski et al., 2006; Malavaki et al., 2011). The expressions of VN, LN, and HS/HP can be changed over the course of different physiological and pathological conditions, resulting in a modified cellular and molecular course of action occurring in the tissue regenerating process. Hence, the aim of this paper was to investigate the influence of the apitherapeutic agent on LN, VN, and HS/HP content changes during experimental burn healing.

2 Materials and methods

2.1 Therapeutic agents

Propolis formulation (apitherapeutic ointment) accepted by the National Institute of Hygiene (certificate number: HZ/06107/00; date: Nov. 4, 2000). Dermazin—1% (0.01 g/ml) silver sulfadiazine (SSD) cream, Sandoz/Lek, Poland.

2.2 Tissue materials

The study protocol was approved by the Ethics Committee of the Medical University of Silesia, Poland. Four 16-week-old domesticated pigs were chosen for the evaluation of wound repair because of the many similarities of pig skin to human skin. Seventy-two contact burn wounds were inflicted according to the methods proposed by Hoekstra et al. (1993) and Brans et al. (1994). Pigs were housed according to the Good Laboratory Practice (GLP) Standards of Polish Veterinary Law. Animals were divided into control (n=2) and experimental (n=2) groups. In the control group wounds were treated with physiologic saline (NaCl) to observe the healing process occurring without management (one animal) or with a propolis vehicle in order to exclude its possible effect on the propolis properties (another animal), twice a day, throughout 21 d. In the experimental group, burns were treated with propolis (one animal) or SSD (another animal), twice a day, for 21 d. Biopsies, in three replications, were taken from healthy skin at Day 0 and from the wound bed on post-burn Days 3, 5, 10, 15, and 21.

After burn infliction, thermally damaged tissues were rinsed with an antiseptic agent and then treated with propolis, SSD, propolis vehicle, and NaCl, respectively. In the case of burn wounds treated with the propolis, SSD, and propolis vehicle, the wound surface was covered with 0.50–0.75 cm layer of topically applied experimental agent. The wounds were then covered with a woven cotton material. The wounds left by the biopsy were covered with collagen dressing.

2.3 Extraction and assay of tissue VN and LN

Tissue samples, after homogenization with acetone (30000 r/min, 4 °C for 30 min) and weighing, were treated with 2 mol/L urea solution in 0.05 mol/L Tris-HCl buffer (pH=7.2) containing 0.2 mol/L NaCl,