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Influence of gender on epithelial host defence peptide gene expression under non-infected and infected conditions: A basic medical research study

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ABSTRACT

Bacterial resistance against conventional antibiotics is increasing. This introduces challenges, for example, in the treatment of infected surgical wounds. Host defence peptides (HDP), which are endogenous peptide antibiotics, show broad-spectrum antimicrobial effectiveness. They protect the organism against pathological microorganisms. Synthetic HDP might supplement or even become alternatives to conventional antibiotics. Knowledge of their quantities under physiological and pathophysiological conditions is therefore required. The influence of gender on HDP expression is unknown. This study evaluates whether gender influences HDP expression in infected or healthy epithelium.

Expression levels of HDP human beta-defensin (hBD)-1, -2 and -3 and psoriasin (S100A7) were analysed, by using real-time polymerase chain reaction, in samples of epithelium from infected surgical wounds (n = 20) and healthy epithelium (n = 14) from the neck in a basic medical research study (analytic observational design).

The results demonstrated a significantly elevated expression of *hBD-2*, *hBD-3* and *psoriasin* ($P = 0.001$ each) in infected epithelium compared with healthy epithelium. No difference in HDP expression levels was evident between samples from female and male patients, either within infected samples or within healthy epithelium samples.

Thus, gender does not affect the cutaneous expression of the investigated HDP. This is fundamental knowledge for the study and potential use of HDP derivates as alternative antibiotic substances.

1. Introduction

Infections of surgical cutaneous wounds can present a challenging task for the treating surgeon. Bacterial resistance to antibiotics is increasing, with a growing proportion of wound infections with gram-positive bacteria being reported (Wilson, 2003; Gould, 2008). Research is therefore being aimed at the identification of innovative antimicrobial therapeutic strategies (Livermore, 2004).

In this context, the possibility of reducing microbial infestation in surgical wounds by effector molecules of the innate immune system has become a scientific focus. This has been initiated by the identification of endogenous peptide antibiotics in the skin, the so-called host defence peptides (HDP) (Steinstraesser et al., 2008). These exhibit broad-spectrum antimicrobial activity against bacteria and viruses and fungi (Fulton et al., 1997; Schittek et al., 2008). Human beta-defensins (hBD), which are a subgroup of HDP, form an important class of effector molecules of the innate immune system. Numerous studies have reported the important role of hBD in wound healing (Steinstraesser et al., 2008; Kesting et al., 2010). In the case of epidermal injury, HDP can contain microbial infestation in the wound within hours, or for prolonged phases, if necessary (Gallo and Huttner, 1998). HDP possess the

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capability of disrupting microbial membranes by the formation of voltage-dependent ion channels (Lehrer et al., 1989; Kagan et al., 1990; Michalek et al., 2009).

As previously described, cutaneous human beta-defensin-1 (hBD-1) synthesis is predominantly localized in keratinocytes, skin glands, and in $CD4^+$/$CD8^+$ T cells. hBD-1 is effective against gram+ and gram− bacteria. Human beta-defensin-2 (hBD-2) is produced in keratinocytes, glandular cells, mast cells and $CD4^+$/$CD8^+$ T cells. hBD-2 is effective against gram+ and gram− bacteria and against fungi. Human beta-defensin-3 (hBD-3) synthesis is localized in keratinocytes, monocytes and $CD4^+$ T cells. It is effective against gram+ and gram− bacteria and against fungi (Harder et al., 1997; Stoeckelhuber et al., 2006, 2008; Schittek et al., 2008; Steinstraesser et al., 2008; Kesting et al., 2010). Psoriasin (S100A7), which is another notable HDP, is produced predominantly in keratinocytes and is effective against gram+ and gram− bacteria. Its name is derived from its original identification in skin lesions of psoriatic patients, in which it has been shown as being up-regulated (Madsen et al., 1991; Fulton et al., 1997; Schittek et al., 2008; Steinstraesser et al., 2008). As described above with respect to hBD, psoriasin permeabilizes microbial membranes by permeabilization. It has further been reported to function as a chemoattractant for leukocytes (Wolf et al., 2008; Michalek et al., 2009).

Several studies have discussed the usefulness of HDP or synthetic HDP derivates as alternative anti-infective treatment modalities (Lipsky et al., 2008). The use of HDP derivates (as single substances or in combination with conventional antibiotics) against cutaneous infections has been tested *in vitro*. Interestingly, an increase in bactericidal activity has been reported by some authors, although a simultaneous increase in unspecific cytotoxity has also been observed (Vaara, 2009). Therefore, research has been aimed at the identification of less cytotoxic HDP derivates (Rennie et al., 2005). However, no such substance is available for clinical use as yet.

Although the antimicrobial benefits of HDP have been extensively highlighted in the literature, current research is also demonstrating potential negative effects, for example, of hBDs in development of atopic dermatitis (Chieosilapatham et al., 2017). Another new interesting development in the field of HDP research is their potential use as anti-biofilm agents. Biofilm formation is a problem with special relevance to dentistry and oral and maxillofacial surgery. Conventional antibiotics show limited effect on biofilm formation. Research therefore is presently aimed at the design of novel antimicrobial strategies on the basis of HDP (Chung and Khanum, 2017). However, basic research is still necessary and, to our knowledge, no substances on HDP basis are available for clinical use yet.

An extensive knowledge of the molecular quantities of the HDP at the gene expression level under physiological and pathological conditions is necessary if these effector molecules are to be used as new anti-infective substances in the future.

To date, no quantitative investigation of potential differences in the expression of hBD and psoriasin between female and male patients has been conducted. However, knowledge of differences in HDP expression between male and female patients is one of the fundamental prerequisites for the possible future use of new antimicrobial substances based on HDP derivates.

2. Materials and methods

2.1. Patients and specimens

We have investigated the expression levels of HDP *hBD-1*, *-2*, *-3*, and *psoriasin* in samples of healthy epithelium (n = 14; 6 female, 8 male) and of inflamed surgical wounds (n = 20; 9 female, 11 male) by means of real-time reverse transcription polymerase chain reaction (RT-PCR) in order to test the primary hypothesis that epithelial HDP expression is different between female and male human individuals (null-hypothesis: epithelial HDP expression is not different between female and male human individuals).

We investigated samples from female and male patients at the Maxillofacial Unit of the University Hospital of the Technische Universität München (Munich) in a basic medical research study (analytic observational design). The methods were approved by the local ethics committee (No. 212108) and are in accordance with the WMA Declaration of Helsinki (as revised 2013). All of the patients gave written informed consent.

Healthy tissue samples were collected from excess tissue from cosmetic surgery or residual flap donor site tissue or surgical approaches during neck dissection procedures (Fig. 1). Inflamed tissue samples were collected from infected surgical wounds after neck dissection procedures (Fig. 2). A wound was regarded as inflamed and was included in the study when clinical signs such as redness, swelling and pain were evident and when a dehiscence occurred.

Each sample was placed in RNA*later*® solution for RNA stabilization (Qiagen, Hilden, Germany) and stored at −80 °C for PCR analysis.

2.2. RNA isolation and reverse transcription

Samples were homogenized in a rotor-stator system (Miccra, ART Prozess-und Labortechnik, Muellheim, Germany). Total ribonucleic acid (RNA) was isolated by using the RNeasy® Protect Mini

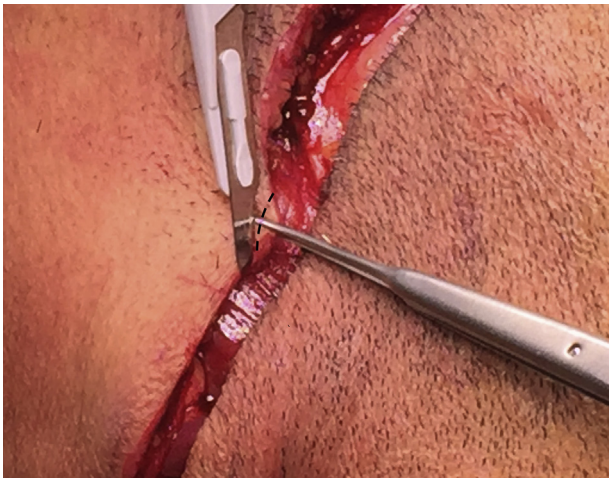

Fig. 1. Acquisition of healthy tissue sample during operation. The dashed line indicates incision.

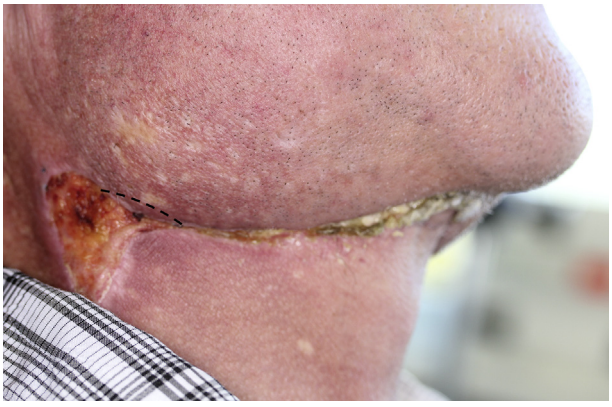

Fig. 2. Sample acquisition from infected wound margins. The dashed line indicates incision.