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Oxidative stress and poly-ADP-ribosylation in normal skin and wound healing

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INTRODUCTION

Oxidative stress, antioxidant systems

Research on oxidative stress is experiencing a renaissance. A wealth of research has demonstrated its role in many diseases. Oxidative stress-induced PARP activation is also implicated in many pathophysiological processes. Oxidative stress is an imbalance between oxidative processes in the body and the so-called antioxidant systems that prevent them, in favour of the former. Oxidative stress can be caused by increased free radical production, but also by reduced degradation, i.e. abnormal functioning of antioxidant systems.

Free radicals can be generated in cells under physiological conditions (endogenous oxidants) and in response to external toxic effects (exogenous oxidants). Radicals can be not only harmful but also essential in certain processes. Therefore, the body does not strive to eliminate free radicals completely, but to maintain an appropriate pro- and antioxidant balance. Among **endogenous oxidants,** ROS are prominent, being involved in physiological processes, play an important role in cell signalling and homeostasis, and non-specific defence against pathogens, but are also produced in high amounts in inflammation. The best known ROS are superoxide (O2--) and the hydroxyl radical (-OH), hydrogen peroxide (H2O2). The best known reactive nitrogen derivatives (RNS) are nitric oxide (NO--) and peroxynitrite (ONOO-). Nitric oxide (NO--) is an important cellular signal transduction molecule, but it also plays a fundamental role in neurological, physiological and immunological processes, and in the regulation of the circulatory system. During inflammatory processes, the reaction of overproduced superoxide anion with NO leads to the formation of highly reactive peroxynitrite (ONOO-), which can damage macromolecules, induce DNA breaks and affect the function of signalling pathways. Nitrotyrosine, formed by the nitration of tyrosine, indicates the presence of peroxynitrite produced in vivo. In addition to endogenous oxidants, a number of external toxic factors can initiate or enhance oxidative processes (**exogenous oxidants**), such as UV radiation, air pollution (cigarette smoke), ionising radiation.

In order to maintain the balance of oxidative molecules produced in the body under physiological conditions, the body has developed a specific protective, scavenging, **antioxidant system**. There are three levels of antioxidant protection. The first level is represented by antioxidant enzymes, which assist in radical scavenging reactions or can themselves neutralise ROS, and the second level by low molecular weight antioxidants, which stop free radical chain reactions that have already started (e.g. The third line of the antioxidant defence system is the

removal of damage that has already occurred, repairing the defective parts (e.g. DNA repair enzymes, heat shock proteins, chaperones).

Today, there is almost no disease or condition in which the damaging effect of free radicals has not been demonstrated, e.g. in arteriosclerosis, ischaemia/reperfusion injury, inflammation, tumours, diabetes. Direct in vitro analysis of free radicals in tissues, cells and body fluids is difficult and expensive due to their very short half-life (electron spin resonance, chemiluminescence), and therefore for routine purposes, more stable molecules, altered proteins, lipids, DNA or their stable metabolites (nitrate, nitrite), which are transformed by reaction with free radicals, are detected. Oxidative stress can also be investigated by detecting and measuring elements of the antioxidant system.

Poly-ADP-ribosylation

The pathogenetic role of reactive oxygen and nitrogen intermediates in most diseases is now well understood. A late step in the ROS/RNS-induced tissue damage pathway is the enhancement of poly(ADP-ribose) (PAR) metabolism, thus providing a broad therapeutic window for clinical intervention. Poly-ADP-ribosylation is a complex regulatory mechanism. Poly-ADP-ribose is one of the most abundant proteins in the nucleus. During poly-ADP-ribosylation, poly(ADP-ribose) polymerases (PARP) cleave NAD+ into nicotinamide and ADP-ribose and synthesize long, branched (ADP-ribose)n polymers from these by coupling them to the glutamate side chain of their respective acceptor proteins. The PAR polymers are degraded by poly(ADP-ribose) glycohydrolase (PARG) and ADP ribosyl protein lyase.

The biological role of poly-ADP-ribosylation

Since PARP-1 is abundant in the nucleus, it can be activated immediately and in large quantities in response to DNA damage. The primary function of PARP-1 is to maintain genome integrity and repair DNA damage. In physiological states, poly-ADP-ribosylation and the interaction of PARP-1 with other proteins affect chromatin structure, play a role in DNA repair, genome organisation and maintenance of genome stability, and regulate replication, transcription, proliferation, differentiation, metabolism and cell death (necrosis/apoptosis). PARP inhibition impairs base excision repair (BER), which is responsible for the excision and replacement of mutagenic bases. Interventions to inhibit poly-ADP-ribose metabolism, PARP enzyme inhibition (e.g. nicotinamide, benzamide, 3-aminobenzamide) and PARP gene-

deficient animal models have shown that PARP overactivation is involved in a number of oxidative stress-mediated disease processes, and PARP inhibitors may be an effective therapy for these pathological conditions

Poly-ADP-ribosylation in the skin

Hinshaw et al. were the first to demonstrate that PARP activation in the skin also occurs in response to sulphur mustard (a cytotoxic DNA-damaging agent). Later, Farkas et al. described the role of PARP activation in UV exposure-induced burns. Szabó and co-workers investigated the cytotoxic effect of peroxynitrite in HaCaT cells and the presence of a peroxynitrite-DNA damage-PARP activation pathway in late-type contact hypersensitivity. Flower and his group investigated the role of PARylation in the effect of UV light on keratinocytes. The presence of PAR in skin tumours has also been investigated. Increased oxidative stress in tumour cells or hypoxia of tumour cells can also cause PARP activation, as indicated by intense PAR staining of blood vessels around the tumour. The significance of PAR detection is that PAR metabolism may be an important target in the adjuvant treatment of melanoma malignancies. Our group was also able to detect the presence of PAR in melanoma malignancies, with the intensity of positivity correlating with the depth of tumour invasion (Clark stage) and thickness (Breslow index). As clinical trials with PARP inhibitors are already underway in various tumours, the determination of PAR content may help to predict the efficacy of antitumour therapy with PARP inhibitors.

Wound healing process in physiological state, acute wounds

Wound healing is a complex multi-phase process in which the skin and subcutaneous tissues recover from injury. Disruption of this complex regulatory process can lead to prolonged wound healing. Common features of various chronic wounds are hypoxia, persistent inflammation, bacterial colonisation and altered stress response.

Normal wound healing process

Normal wound healing takes 6-7 days. In the *haemostasis phase*, coagulation and vasoconstriction occur, with platelets playing a predominant role. Fibrin activation, clot formation reduces bleeding, growth factors (EGF, TGF alpha, beta, PDGF) produced by platelets, endothelial cells, keratinocytes trigger the subsequent phase of wound healing. In the second *phase of inflammation*, which lasts 1-3 days, various immune cells invade the wound, with neutrophils and macrophages playing the main role, clearing away debris and bacteria. In

addition to these, endothelial activation, activation of pro-inflammatory cytokines (e.g. IL-1β, IL-6, TNFα, IFN), growth factors (PDGF, TGFβ), proteolytic enzymes, ROS/RNA species also aid the process. The third granulation and profiling phase lasts from day 3 to week 3. In this phase, fibroblast activation leads to connective tissue accumulation, extracellular matrix production, neoangiogenesis, lymphangiogenesis. Several cytokines, growth factors, such as transforming growth factor-β family, interleukin (IL) family and angiogenesis factors (e.g. VEGF-vascular epidermal growth factor) are involved in the process. The final step of wound healing is the *epithelialisation or remodelling phase*, which lasts from day 7 to about week 6. Through keratinocyte migration, proliferation and differentiation, the epidermal barrier is restored, the wound is exfoliated, and the type III collagen characteristic of granulation tissue is gradually degraded and replaced by type I collagen for strength.

Pathological, prolonged wound healing, chronic wounds

The complex process of wound healing can be disrupted at many points and can lead to the development of non-healing chronic wounds or abnormal scars (e.g. hypertrophic scars, keloids). A chronic wound is defined as one in which complete wound healing does not occur within 6 weeks, in which case there is usually persistent inflammation, usually followed by a persistent granulation, proliferative stage. In chronic wounds, epithelialisation, remodelling is also prolonged, partial or does not occur. Wound healing is delayed by a number of factors, the most common being impaired local circulation, hypoxia, venous stasis, tension at the wound edges, infections, chronic inflammation, nutritional problems, malnutrition, immunosuppression or metabolic dysregulation, especially diabetes. In addition to numerous studies, our own studies have shown that oxidative stress plays a role in the pathogenesis of non-healing wounds.

Oxidative stress in wound healing, the role of antioxidants in wound healing

A growing body of research has demonstrated the role of oxidative stress in wound healing. ROS play an important role in this process, their small amounts being an essential mediator of intracellular signalling. High levels of ROS negatively affect wound healing. In most studies, ROS levels in wounds are determined indirectly by analysing the oxidation products of lipids, proteins. A sharp increase in the concentration of 8-isoprostanes, a major product of lipid peroxidation, in chronic wounds and the presence of increased protein carbonylation induced by oxidative stress have been described. There is also evidence that

nitrotyrosine, indicative of the presence of peroxynitrite, may also be increased in wounds. Decreased levels of low mass antioxidants (e.g. vitamin E, vitamin C, glutathione) have been shown to be associated with impaired wound healing in a number of animal studies, and have been described in diabetic animal models. In addition to low-molecular-weight antioxidants, several ROS antioxidant enzymes (SOD, catalase, glutathione peroxidase, peroxiredoxins, heme oxygenases) are also key in regulating cellular redox balance... The role of SODs in wound healing has been investigated in an animal model of ischemic wounding, where they promoted healing.

The possible role of poly-ADP ribosylation in wound healing

Diabetes mellitus and ischaemia are the two main aetiologies of non-healing wounds of the lower limb. Hyperglycaemia from diabetes and oxidative stress from ischaemia activate PARP-1, which is known to affect a number of cellular functions in addition to its role in DNA damage repair. There is evidence that both diabetes mellitus and ischaemia induce PARP-1 and that several PARylation-related responses (oxidative stress response, expression of inflammatory cytokines and chemokines, cell proliferation and migration) play a very important role in wound healing. Many details are still unclear regarding the role of PARP-1 in diabetic wounds. PARP-1 also contributes to the production of various proinflammatory mediators during the ***inflammatory phase*** of wound healing. Inhibition of PARP-1 by 3-aminobenzamide (3-AB) has been shown to attenuate the oxidative/nitrosative stress response and accelerate wound healing. During the ***proliferation phase***, PARP inhibition may be used to enhance angiogenesis and improve granulation tissue formation. There are several data that PARP inhibitors promote keratinocyte proliferation, migration, and angiogenesis was enhanced in PARP-1 knockout wound healing models. Inhibition of PARP during the ***remodelling phase*** of wound healing can reduce scarring in animal models.

AIMS

One of the main objectives of our studies was to investigate the role of polyADP-ribosylation (PARylation) in skin physiology and wound healing. In our work, we specifically investigated the role of oxidative/nitrosative stress-stimulated PARylation. We examined whether the peroxynitrite- DNA damage- PARP activation pathway is present in the wound healing process in acute and chronic wounds. We also aimed to characterize the redox environment in normal skin and in the wound healing process using oxidative stress markers and antioxidant capacity measurements.

In our research, we sought to answer the following questions:

1. Is PAR /poly(ADP-ribose)/ detectable in normal skin, and if so, in which skin cells?

2. Is peroxynitrite produced by the reaction of NO and superoxide detectable in histological samples of chronic wounds and, if so, is there a difference in its presence compared to normal skin?

3. Is PARP activation detectable in chronic wounds?

4. If the peroxynitrite-DNA damage- PARP activation pathway is detectable during wound healing, does it have a role in the wound healing process, does it affect prolonged wound healing?

5. Is oxidative stress biomolecule damage detectable in the wound fluid of acute and chronic wound patients, if so, is there a difference in their presence?

6. Is there a difference in antioxidant capacity in the wound fluid of acute and chronic wound patients?

7. Is there evidence of biomolecular damage and antioxidant capacity indicative of oxidative stress in the serum of patients with acute and chronic wounds and, if so, is there a difference in their presence?

8. Is there a difference in the production of inflammatory mediators in acute and chronic wounds, and if so, is the difference also manifested in serum and wound fluid?

9. Are there correlations between the measured data and, if so, what are the relationships?

MATERIALS AND METHODS

The materials and methods used in our work are not described in this thesis for reasons of space, but can be found in Chapter 3 of the thesis.

RESULTS

1. Immunohistochemical studies in normal skin and chronic wounds:

1.1. Analysis of poly(ADP)ribose /PAR/ in normal skin

To detect poly(ADP)ribose polymerase (PARP) activity, the presence of poly(ADP)ribose (PAR), the end product of the enzyme, was assayed in normal skin by immunohistochemistry. In normal skin, PAR can be detected in many areas: in the epidermis in keratinocytes, in hair follicle cells, in the dermis in endothelial cells, and in other cells of the vascular wall, sebocytes, sebaceous gland cells and adipocytes of subcutaneous adipose tissue. PAR positivity was also present in melanocytic naevus.

1.2. Nitrotyrosine and PAR in chronic wounds

Samples were obtained from the edges of ulcers due to chronic venous insufficiency. Sections of these tissues were subjected to immunohistochemistry for the detection of nitrotyrosine and PAR. Normal skin was used as control. Both nitrotyrosine and PAR were more intensely present in venous wounds compared to controls. In normal skin samples, weak nitrotyrosine and PAR staining was detectable or no staining was detected. Semi-quantitative analysis showed significantly elevated nitrotyrosine and PAR staining in the wound bed area and ulcer margins compared to normal skin.

2. Analysis of wound fluid and serum samples

To characterise the redox homeostasis present in the wound healing process, we examined the presence of reactive oxygen and nitrogen intermediates (ROS/RNS) and antioxidant systems (root scavenging capacity and glutathione levels). Our studies were performed on wound fluid and serum samples.

2.1 Measurement of biomolecule damage and antioxidant levels in wound fluid in patients with acute and chronic wounds

There was no significant difference in protein oxidation and tyrosine nitration of proteins in wound fluid samples from the two groups of patients, i.e. acute and chronic wound patients. The rate of lipid peroxidation was significantly higher in chronic ulcer wound fluid samples compared to acute. Furthermore, the root scavenging capacity and the levels of the major antioxidant, glutathione, were significantly higher, with chronic wounds having a higher antioxidant capacity compared to acute ones.

2.2 Determination of biomolecule damage and measurement of antioxidant levels in serum of patients with acute and chronic wounds and healthy controls

There was no significant difference in serum samples from wound patients between those with acute and chronic ulcers. Protein carbonyl and lipid peroxidation were slightly but significantly lower in the sera of patients with chronic wounds compared to healthy controls. Levels of lipid peroxidation products were also lower in the sera of patients with acute wounds compared to controls.

There was no significant difference in protein tyrosine nitration in the sera of patients with wounds compared to healthy controls, but lower levels of nitrotyrosine were measured in the sera of patients with chronic wounds compared to those with acute wounds.

There was no significant difference in the root catching capacity and antioxidant levels in sera from patients with acute and chronic wounds compared to healthy controls.

2.3. Analysis of markers in the wound healing process in wound fluid and serum from patients with acute and chronic wounds and healthy control serum

Levels of lactate dehydrogenase (LDH) activity, IL-8 levels indicative of granulocyte outflow and infiltration, TNF-alpha levels indicative of inflammation and VEGF levels indicative of vascularisation were significantly higher in the wound fluid of patients with chronic wounds compared to acute wounds. But these biomarkers were not detectable in patients' serum.

3. Finding correlations between measured biochemical parameters

Pairwise correlation was performed for all measured biochemical parameters. Significant correlation was found between protein carbonyl levels and lipid peroxidation values in the serum of chronic wound patients. VEGF and TNF-alpha levels in chronic wound fluid samples were also significantly correlated. Furthermore, in wound fluid samples from patients with acute wounds, a positive correlation was found between lipid peroxidation and glutathione levels, while an inverse correlation was found between tyrosine nitration and root catching capacity.

4. Correlation analysis between wound fluid/serum biomarkers and patient age

When examining the difference between the redox environment of acute and chronic wounds, the fact that the patient population we studied has a different age distribution should not be ignored. The average age of our patient population was 66.5 years for patients with chronic wounds and 45.5 years for patients with acute wounds. Since the redox environment can change with age, we used correlation analysis to exclude the possibility that the differences between the two study groups were due to differences in mean age. In fact, some of the redox and inflammatory parameters that we measured (serum ABTS scavenger activity and TNFα, IL-8 and VEGF levels in wound fluid) were positively correlated with age in all patient groups.

DISCUSSION

1. Poly-ADP-ribosylation in normal skin

We first demonstrated the presence of poly-(ADP-ribose) in normal skin. The expression of PARP-1 in the main epidermal cells, keratinocytes, has been described previously. In addition, several research groups have demonstrated the dominant expression of PARP-1 in cultured primary keratinocytes and HaCaT cells. PARP-1 activation in keratinocytes has also been associated with inflammatory skin diseases and sunburn. PARP-1 has also been shown to regulate the expression of inflammatory cytokines and chemokines (IL-1, TNF- α, MIP-1a, MIP-2, MCP-1, etc.) and oxidative stress-induced cell death. In the present studies, we have shown that PAR may also serve as a signalling molecule in keratinocytes, as the polymer is detectable in keratinocytes of healthy skin. However, further studies are needed to show whether PAR synthesis in keratinocytes is induced by DNA breaks or by processes independent of DNA breaks.

Intense PAR staining was present in hair follicle cells. This may be due to the rapid cell cycle, supported by the fact that PARylation plays a role in the regulation of proliferation. PAR polymers were also detected in sebocytes and adipocytes, which may suggest a new role for PAR in these lipid-accumulating cell types. The role of PARP-1 in adipocytes has been suggested, but the presence of PARP-1 in mature adipocytes was first described by our group. In recent years, several scientific publications have reported the role of PARP in fat metabolism. Based on literature data, it is likely that the binding of PARP-1 to active nuclear receptors is triggered by DNA strand breaks during receptor activation, and thus this probably explains the presence of PAR in these cells.

2. Oxidative, nitrosative stress in acute and chronic wounds

Wound healing is a coordinated series of biochemical events. ROS/RNS species play an essential signalling role in the healing process, for example in cell proliferation and angiogenesis, and contribute to the defence against pathogens. However, increased production of reactive intermediates can lead to an imbalance in redox homeostasis, which can lead to prolonged wound healing in e.g. diabetes, chemotherapy, radiotherapy or steroid treatment. It is not fully understood how the redox environment changes in acute and chronic wounds and

whether they differ. It is also not known how the redox environment changes in injured tissues and how this is reflected in the serum. To better understand this process, we aimed to characterize the redox environment in acute and chronic wounds.

Our studies looked at whether oxidative stress markers such as protein carbonylation, lipid peroxidation, tyrosine nitration are present in acute and chronic wounds and whether there is a difference in their presence in serum and tissue, wound fluid, and also looked at antioxidant levels. Furthermore, we looked at how important key molecules in wound healing are present in different wounds and how they are expressed in serum. We could detect protein carbonylation in wound fluid from both acute and chronic wounds, but there was no significant difference in intensity between acute and chronic wounds. Lipid peroxidation was slightly more intense in the chronic wound fluids, whereas there was no difference in tyrosine nitration between wound fluids from acute and chronic wounds. However, the antioxidant profiles of acute and chronic wounds were significantly different: chronic wounds had higher radical scavenging activity and higher GSH content compard to acute wounds.

3. Key biomolecules in wound healing in acute and chronic wounds

LDH activity, levels of VEGF, a key mediator of vascularisation, and levels of the major granulocyte recruiting factor and inflammatory cytokine IL8 and TNFα, indicative of the extent of tissue damage, were higher in the wound fluid of chronic wounds compared to acute wounds. Together, these data suggest a more pronounced inflammatory environment in chronic wounds, which is likely to be associated with higher ROS/RNS species production. The compensatory overproduction of antioxidants may explain why increased ROS/RNS production does not cause increased biomolecular damage. The slightly but significantly higher lipid peroxide levels in chronic wound fluids probably indicate that lipids are more vulnerable targets than proteins.

The effects of molecular events in the affected tissues are often reflected in the blood circulation. Therefore, we investigated whether changes in the redox environment during wound healing could be detected in patients' serum. We found no significant differences in the levels of biomolecular damage markers such as root scavenger activity, glutathione levels, protein oxidation and lipid peroxidation in serum samples from patients with acute and chronic wounds and the control group. This result can be explained by the fact that the redox modifications remain localized in the wound area and the wound environment, but do not cause significant differences systemically. It should be noted that lipid peroxidation products appear

with lower values in the sera of patients with both acute and chronic wounds compared to controls, while significant differences were found in wound fluid samples between the two wound groups. It is likely that the wound fluids directly reflect the level of biochemical processes taking place in the tissues, while serum levels are more affected by the clearance mechanism.

4. Correlations between measured parameters, correlation studies

We examined whether there is a correlation between the measured biochemical parameters. Pairwise correlation analysis of the measured biochemical parameters revealed significant correlation in some pairs. It should be noted that the positive correlation between two biomolecular markers, namely lipid peroxides and protein carbonyls, may suggest that the same type of oxidative redox environment induces these two modifications in the sera of patients with venous ulcers. An inverse correlation between root trap activity and nitrotyrosine formation indicates that root traps may inhibit tyrosine nitration in the sera of patients with acute wounds. The positive correlation between lipid peroxidation and glutathione levels in sera of patients with acute wounds is somewhat surprising. In a number of oxidative stress-related conditions, it has been shown that lipid peroxidation typically increases while glutathione levels fall in inflammatory tissues. Other studies have reported that no parallel changes in glutathione and lipid peroxide levels have been observed, which may suggest that there is likely a more complex relationship between these parameters.

It is known that VEGF expression is primarily regulated by hypoxia, but there is literature evidence that inflammatory cytokines such as TNFα and IL-1β and oxidants such as H2O2 can also increase VEGF expression. Previously, H2O2-induced VEGF expression in skin has been shown and its wound healing promoting effects have been described. Induction of VEGF by H2O2 appears to be independent of the hypoxia pathway, but can be inhibited by the thiol antioxidant N-acetylcysteine. In our chronic wound fluid samples, TNFα levels correlated with VEGF levels. Thus, we hypothesize that the inflammatory and oxidative signal induces VEGF expression in chronic wounds.

While examining the difference between the redox environment of acute and chronic wounds, we should not ignore the fact that our study population has a different age distribution. The average age of our patient population was 66.5 years for patients with chronic wounds, while the average age of patients with acute wounds was 45.5 years. The redox environment

can change with age, raising the possibility that some of the differences between the two study groups were because they differed in mean age. However, for several parameters we can rule out that the measured difference is due to differences in average age. In fact, some of the redox and inflammatory parameters measured in this study (serum scavenger capacity /ABTS scavenger activity/ and wound fluid TNFα, IL-8 and VEGF levels) were correlated with age in all patient groups. Of these parameters, serum ABTS activity showed no difference between study groups. Since the other oxidative damage parameters did not show age dependence, we think it unlikely that the studied signals of redox stress in wound fluids are a consequence of age. On the other hand, TNFα, IL-8 and VEGF levels were positively correlated with age and showed higher values in wound fluid samples from chronic wounds compared to acute wounds. Further studies with similar age groups of patients are needed to confirm or refute the role of age in higher TNFα, IL-8 and VEGF levels in chronic wound fluids compared to acute cases.

5. Limitations of the studies

When interpreting the differences between the two types of wound fluids, it is important to consider the following. While the chronic wounds from which wound fluid was collected were exposed to the external environment (apart from the covering dressing) and had their own microbiome present, the blister fluids from the acute wounds were covered by a blister cap and were sterile.

6. Peroxynitrite- PARP activation in chronic wounds at histological level

Immunohistochemistry shows increased tyrosine nitration in chronic wounds compared to healthy skin, probably due to increased expression of inducible nitric oxide synthetase together with increased superoxide production. Peroxynitrite and hydroxyl radicals are known to cause DNA breakage leading to PARP-1 activation. PARP activation in human chronic wounds has been demonstrated by immunodetection of the enzyme end product poly(ADP-ribose). Increased PARP-1 expression was detected in wounds, especially at the wound edges. Increased PARylation may signal DNA repair following oxidative DNA damage, but may also contribute to cellular damage under increased oxidative stress.

CONCLUSION

Based on our findings, we can make the following observations in response to the questions posed in our target setting:

1. Our studies demonstrated the presence of PAR in keratinocytes, sebocytes, hair follicles, endothelial cells and subcutaneous adipocytes in normal skin, indicating that PAR may regulate physiological functions in these cells.

2. Our data demonstrate that peroxynitrite is generated in human wounds. Immunohistochemistry shows increased tyrosine nitration in chronic wounds compared to healthy skin.

3. PARP activation in human chronic wounds was demonstrated by immunodetection of the end product of the enzyme, poly(ADP-ribose).

4. Increased PARP activation was detected in wounds compared to normal skin, with PAR expression being more pronounced at the wound edges. Increased PARylation may indicate DNA repair following oxidative DNA damage, but may also contribute to cell damage in chronic wounds under increased oxidative stress. Increased PARylation may have a role in prolonged wound healing.

5. Biomolecular damage was measured by oxidative stress markers, protein carbonylation, lipid peroxidation, tyrosine nitration, present in acute and chronic wound fluids. We found a difference in the extent of lipid peroxidation, with significantly higher activity in chronic ulcer wound fluid samples compared to acute, and no significant difference between the two groups in the other parameters.

6. Root scavenging capacity and the main antioxidant, glutathione, levels were also significantly higher, with chronic wounds having a higher antioxidant capacity compared to acute. The antioxidant profile in acute and chronic wounds was significantly different: chronic wounds had higher root scavenging activity and higher GSH content compared to acute wounds. The compensatory overproduction of antioxidants may explain why increased ROS/RNS production does not cause increased biomolecular damage.

7. No significant differences were found in the levels of biomolecular damage markers in serum samples from patients with acute and chronic wounds and the control group, nor in root

scavenging activity, glutathione levels, protein oxidation and lipid peroxidation. This result can be explained by the fact that redox modifications remain localized in the wound area and wound environment, but do not cause significant differences systemically in patients with acute and chronic wounds.

8. LDH activity, VEGF, IL-8 and TNFα levels were higher in the wound fluid of chronic wounds compared to acute wounds. This suggests an inflammatory environment in chronic wounds, which is likely to be associated with higher ROS/RNS species production.

9. Pairwise reciprocity was performed for all measured biochemical parameters. Significant correlation was found between protein carbonyl levels and lipid peroxidation values in the serum of chronic wound patients. Furthermore, in chronic wound fluid samples, VEGF and TNF-alpha levels were also significantly correlated, which may suggest that the inflammatory and oxidative signal induces VEGF expression in chronic wounds. Furthermore, we found a positive correlation between lipid peroxidation and glutathione levels in wound fluid samples from patients with acute wounds. Of the redox and inflammatory parameters we measured (serum ABTS scavenger activity and TNFα, IL-8 and VEGF levels in wound fluid), we found a positive correlation with age in all patient groups.

SUMMARY

Research on oxidative stress is experiencing a renaissance. A wealth of research has demonstrated its role in many diseases. Oxidative stress-induced PARP activation is also implicated in many pathophysiological processes.

One of the main aims of our studies was to investigate the role of PARylation in skin physiological processes and wound healing. In our work, we specifically investigated the role of oxidative/nitrosative stress-stimulated PARylation. We examined whether the peroxynitrite-DNA damage- PARP activation pathway is present in the wound healing process in acute and chronic wounds. We also aimed to characterize the redox environment in normal skin and the wound healing process using oxidative stress markers.

In our studies, we demonstrated the presence of PAR in keratinocytes, sebocytes, hair follicles, endothelial cells and subcutaneous adipocytes in normal skin, indicating that PARP may regulate physiological functions in these cells.

Oxidative stress is known to play a role in the complex process of wound healing, but many details remain to be explored. We have characterised the redox environment of acute and chronic wounds. Based on our results, it is clear that the redox environment in chronic human wounds differs from that in acute wounds, as reflected by intense inflammation and higher antioxidant levels. Further analysis of the role of specific ROS species as well as redox signalling mechanisms may provide targeted therapeutic options for the treatment of delayed wound healing in the future.

The question arises as to whether a detailed redox and inflammatory biomarker profile of wound fluids or sera will be able to provide predictive biomarkers for the identification of non-healing wounds. Peroxynitrite production and PARylation are also present in chronic wounds and are likely to contribute to tissue damage. Further studies are needed to clarify the role of these processes in normal and pathological wounds, and we have already begun our investigations.

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Candidate: Edina Bodnár
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List of publications related to the dissertation

1. **Bodnár, E.**, Bakondi, E., Kovács, K., Hegedűs, C., Lakatos, P., Robaszkiewicz, A., Regdon, Z., Virág, L., Szabó, É.: Redox Profiling Reveals Clear Differences between Molecular Patterns of Wound Fluids from Acute and Chronic Wounds.
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3. **Bodnár, E.**: Korunk népbetegsége: a körömgomba. Szisztémás antimikotikum vagy körömlakk segíthet?
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