”Changes in the surface area of pressure ulcers and gene expression of selected metalloproteinases and transforming growth factor in human pressure ulcers after application high voltage electrostimulation”

Introduction: Wound healing is a complex process aimed at repairing tissues and restoring skin functions. During wound healing, the functioning of the cells involved is regulated by mediators of the wound healing process - chemokines, cytokines, enzymes, hormones and growth factors.

Chronic wounds that do not heal properly are a serious health problem that affects many millions of people around the world. There is then too much activity of pro-inflammatory cytokines and proteases, with a simultaneous reduced effect of anti-inflammatory cytokines and protease inhibitors. Additionally, reduced activity of growth factors and their receptors can be observed in the membranes of cells involved in wound healing.

One of the most common chronic wounds are bedsores, which affect people who have suffered damage to the central nervous system, people staying in long-term care facilities and patients in intensive care units.

Treatment of pressure ulcers is a long-term and expensive process, so methods are being sought that can reduce costs and can be used at home. For this purpose, electrostimulation is increasingly used and its possibilities in the treatment of chronic wounds are being investigated.

The aim: The main aim of the study was to obtain knowledge about the impact of EWN on the healing process of stage II-IV pressure ulcers in people after damage to the central nervous system. The specific objectives were to answer the questions whether anodal and cathodic EWN would contribute to reducing the area of pressure sores after the 1st, 2nd, 3rd and 4th week of treatment and whether anodal EWN and cathodic will cause changes in the gene expression of selected metalloproteinases (MMP-1, MMP-2 and MMP-9) and individual isoforms of transforming growth factor-β (TGF-β1, TGF-β2 and TGF-β3). We also sought answers to the question whether there would be strong and statistically significant correlations between changes in the surface area of pressure ulcers and the expression of MMP-1, MMP-2, MMP-9, TGF-β1, TGF-β2 and TGF-β3 mRNA genes in people with pressure ulcers II- IV degree. The application goal of the study was to indicate the possibility of using anodal and cathodic EWN in the treatment of stage II-IV pressure ulcers in people with injuries to the central nervous system.

Material and methods: The user included 60 people with stage II-IV pressure ulcers, aged 22-78. The participants were randomly divided into 3 groups, 20 people each - a group subjected to anodal ECT (GEA), a group subjected to cathodal ECT (GEK) and a control group (GK). All people were treated in accordance with clinical recommendations. High-voltage electrostimulation performed using double, pointed electrical impulses, the total duration of which is 154 μs and occurs at 100 Hz. The peak value of the current source was 0.36 A. The power intensity was below the excitability threshold of skeletal muscles. To deliver a supplied electric current of 360 μC/s. In the GEA group, the treatment electrode was the anode, in the GEK - the cathode, and in the control group - simulated EWN. Of these 60 people undergoing the study, 41 people (14 people in GEA, 12 in GEK and 15 in GK) agreed to take sections from pressure ulcers and determine the expression of MMP and TGF-β genes.

Results: Both in GEA and GEK the surface area decreased statistically significantly in weeks 2, 3 and 4 of therapy ($p = 0.01$). In the CG, the surface area also decreased, but it was not statistically significant ($p > 0.05$). Changes in the relative expression of MMP-1, MMP-2 and MMP-9 mRNA in all groups did not show statistically significant differences at individual time points (Friedman's ANOVA test for MMP-1: GEA: $p = 0.6065$; GEK: $p = 0, 6675$; GK: $p = 0.0780$; Friedman's ANOVA test for MMP-2: GEA: $p = 0.8071$; GEK: $p = 0.8616$; GK: $p = 0.6918$; Friedman's ANOVA test for MMP-9: GEA: $p = 0.5258$; GEK: $p = 0.2974$; GK: $p = 0.4703$). RE changes of TGF-β1 mRNA in all groups did not change statistically significantly at individual time points (GEA: $p = 0.2574$, GEK: $p = 0.8257$, GK: $p = 0.5165$). Changes in the relative expression of TGF-β2 mRNA in all groups, tested using the Friedman ANOVA test, also turned out to be statistically insignificant (GEA: $p = 0.8071$, GEK: $p = 0.8257$, GK: $p = 0.3365$). GEA, GEK and GK showed no significant changes in the RE of TGF-β3 mRNA as a result of therapy (Friedman's ANOVA test, respectively: $p = 0.1537$, $p = 0.6675$, $p = 0.7928$). Before the intervention, a statistically significant difference was detected between groups in terms of RE TGF-β3 mRNA (Kruskal Wallis ANOVA test: $p(GEA:GEK:GK) = 0.0020$). Kruskal Wallis post hoc analysis showed that before the RE intervention, TGF-β3 mRNA in GEA and GEK was statistically significantly lower than in GK ($p(GEA:GK) = 0.0013$; $p(GEK:GK) = 0.0397$) . Between the experimental groups before the RE intervention, TGF-β3 mRNA did not differ statistically significantly ($p(GEA:GEK) = 0.1940$). After 2 weeks of RE intervention, TGF-β3 mRNA increased statistically significantly in GEK compared to GEA (post-hoc test: $p(GEA;GEK) = 0.0453$), at the same time the change did not differ statistically significantly compared to GK (test post-hoc: $p(GEK:GK) = 0.9130$). In GEA RE, TGF-β3 mRNA after 2 weeks of intervention was still at a statistically significantly lower level than in CG (post-hoc test: $p(GEA:GK) = 0.0008$). After 4 weeks,

the RE of TGF-β3 mRNA in GEA was still statistically significantly lower than in GK (post-hoc test: p(GEA:GK) = 0.0165). In GEK RE, TGF-β3 mRNA was statistically insignificantly decreased compared to the state after 2 weeks of intervention and was no longer statistically significantly higher than in GEA (post-hoc test: p(GEA:GEK) = 0.1190), it was still maintained at a level comparable to GK (post-hoc test: p(GEK:GK) = 0.9990).

Conclusions:

Anodal and cathodic high-voltage electrical stimulation performed for 4 weeks contribute to a similar extent to reducing the area of II-IV degree pressure ulcers in people with damage to the central nervous system. A statistically significant reduction in the area of stage II-IV pressure ulcers in humans occurs already after the second week of use of both anodic and cathodic EWN, and these effects persist at least until the 4th week of treatment.

Cathodic and anodal EWN performed for 2 and 4 weeks do not affect the expression of MMP-1, MMP-2 and MMP-9 genes in stage II-IV pressure ulcers in people with damage to the central nervous system.

Anodal EWN performed for 2 and 4 weeks does not affect the expression of TGF-β1, TGF-β2 and TGF-β3 genes in stage II-IV pressure ulcers in people with damage to the central nervous system. Cathodic EWN performed for 2 and 4 weeks also does not affect the expression of TGF-β1 and TGF-β2 genes, but increases the expression of TGF-β3 genes in stage II-IV pressure ulcers in people with damage to the central nervous system, and this expression after 2 weeks of EWN cathodic is greater than under the influence of anodic EWN.

In patients with central nervous system damage who underwent anodal and cathodal ECT, there will be no strong and statistically significant correlations between the expression of the MMP-1, MMP-2 and MMP-9 genes and changes in the area of grade II-IV pressure ulcers.

In patients with central nervous system damage who underwent anodal and cathodal ECT, there will be no strong and statistically significant correlations between the expression of TGF-β1, TGF-β2 and TGF-β3 genes and changes in the area of grade II-IV pressure ulcers.

Keywords: pressure ulcers, electrostimulation, high-voltage pulsed current, surface area, gene expression, MMP, TGF-β