










The Role of Chronotype of Patients and Cryoextract of Placenta in the Activity of Antioxidant Enzymes in Facial Scar

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Abstract

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BACKGROUND: The chronotypical features of people have a significant impact on the course of reparative processes in the patient's body. Understanding the mechanisms of influence on the course of reparative skin regeneration after planned surgical interventions within the maxillofacial tissues can contribute to improving the provision of medical care to such patients.

AIM: The study was aimed to determine role of chronotype of patients and cryoextract of placenta in the activity of antioxidant enzymes in facial scar.

METHODS: In our study, 60 patients (36 males and 24 females) of the Department of Maxillofacial Surgery of the Poltava Regional Clinical Hospital, Ukraine, aged from 18 to 68 years, who underwent inpatient treatment for planned surgical interventions of the maxillofacial area, were enrolled. At the time of the study, the patients did not have concomitant diseases, but if necessary, all patients could consult by other specialists. The practical section was carried out between September 2019 and August 2021 as follow. According to our study, 60 patients were divided depending on the type of chronotype, which was determined by the Horn-Ostberg questionnaire in Stepanova's modification and the type of using treatment taking into account different ways of using of cryoextract of placenta. The activities of superoxide dismutase and catalase were determined on the 90th, 180th, and 360th days of observation in the forming scar tissue. We used the method of Chevri *et al.* to determine the activity of superoxide dismutase. Catalase activity in homogenates of scar tissue was determined according to the method of M.A. Koroliuk.

RESULTS: It was noted that the enzymatic activity of catalase in the scar homogenate is characterized by a lower dynamics of changes than the activity of superoxide dismutase. Positive dynamics were noted under the conditions of use in the treatment protocol of placenta cryoextract, which was more pronounced in patients with a morning chronotype.

CONCLUSIONS: The most significant changes were observed in the studied indicators of antioxidant protection in patients of the morning chronotype who underwent intrawound administration of cryoextract of the placenta in combination with electrophoresis of this remedy against the background of a standard treatment protocol, especially at earlier terms after surgical treatment.

Introduction

The problem of scar tissue formation after surgical interventions has been relevant for a long time [1]. The formation of cicatrice tissues occurs as a result of the processes of reparative regeneration at the site of the inflammatory process, which occurs as a result of damage to the skin [2]. The features of scars of maxillofacial localization are their visualization and, in addition to functional impairment, cause psychological disorders in patients [3]. Therefore, the medical and social problem of preventing the formation of pathological scars especially in the maxillofacial localization is significant and requires finding ways to solve it [4].

The mechanism of scar tissue formation is influenced by a significant number of endogenous (genetic predisposition, and the presence of certain

general somatic diseases) and exogenous factors (mechanism of wound closure, use of adhesive compositions, and use of certain medicinal products both locally and generally) [5].

The nature of scar tissue formation is influenced by various factors, such as the depth and area of the formed defect, the state of the body's reparative processes. The quality of scar tissue formation depends on the way the wound heals. When the wound is healed by primary tension, the scar tissue has a small volume, after which young connective tissue fibroblastic strands sprout between the cellular elements already in the 1st days after the injury [6]. When the wound heals by secondary tension, the wound undergoes a phase of granulation tissue formation, which causes a significant increase in volume and a change in the color of the newly formed scar. The formation of pathological scars occurs when the deeper layers of the skin are damaged, namely the reticular (mesh) layer of the dermis, where accelerated angiogenesis and collagen accumulation occur [7].

Anatomical localization plays an important role in the choice of scar prevention and treatment methods, as it largely determines their esthetic end result in each specific case [8].

Circadian rhythm is cyclic fluctuations of biological processes occurring in organism, which are normally directly related to the change of time of day. It has been established that the chronotypical features of people have a fundamental impact on the course of reparative processes in the patient's body, play a significant role in regulating biological functions, including sleep-wake preference, body temperature, hormonal secretion, food intake, and cognitive and physical performance. In addition, the compliance of the time of the surgical intervention with a certain chronotype of the patient is of significant importance [9], [10]. It should be noted that planned surgical interventions are mostly carried out in the morning time, thereby determining the possible compliance or non-compliance with the patient's circadian rhythm [11].

It is well known that placental tissue contains a wide range of substances acting on the body: Hormones and hormone-like substances, proteins, polypeptides, nucleic acids, lipids, and vitamins [12]. Experimental data allow us to state that when using the placenta, there is stimulation of the endocrine organs, the liver, the trophicity of the cardiovascular system improves, and the body's reparative ability increases also it ensures trophic processes and protein synthesis, gas exchange, hormone secretion and hormone regulation, blood pressure regulation, blood coagulation, antitoxic function and excretion of metabolites, deposition of biologically active substances, immune regulation, and regulation of lipid peroxidation processes [13]. The use of cryopreserved fragments of the placenta affects the target organs, stimulating their function, increases the body's non-specific resistance to adverse environmental factors and stressful situations. It is important to note that placenta preparations are able to exert their effect at the cellular and subcellular level and are considered a powerful stabilizer of the body's homeostasis. This way the placenta cryoextract can play an important role in all reparative processes of organism including cicatrice tissue formation [14].

At present, there is no preventive scheme that would guarantee the formation of normotrophic scars both in the immediate and distant post-operative period. All the attention of researchers today is focused on biocompatible and bioenergetic materials, which have minimal impact, both in local and general application. Understanding the mechanisms of influence on the course of reparative skin regeneration after planned surgical interventions within the maxillofacial tissues and healing of the postoperative wound by primary tension and the association between rhythm and reparative processes can contribute to improving the provision of medical care to such patients, and form an algorithm for the post-operative prevention of the formation of pathological scars [15].

The study was aimed to determine role of chronotype of patients and cryoextract of placenta in the activity of antioxidant enzymes in facial scar.

Materials and Methods

In our study, 60 patients (36 males and 24 females) of the Department of Maxillofacial Surgery of the Poltava Regional Clinical Hospital, Ukraine, aged from 18 to 68 years, who underwent inpatient treatment for planned surgical interventions of the maxillofacial area, were enrolled. At the time of the study, the patients did not have concomitant diseases, but if necessary, all patients could be consulting by other specialists. The practical section was carried out between September 2019 and August 2021 as follow. Patients were divided depending on the type of chronotype, which was determined by the Horn-Ostberg questionnaire in Stepanova's modification and the type of using treatment taking into account different ways of using of cryoextract of placenta [16]. The surgical interventions were conducted in the morning time in every clinical case. Written informed consent was obtained from all patients enrolled in this study along with anonymization of all data.

Patients were divided into 6 clinical groups, which are shown in Table 1.

To assess the state of reparative skin regeneration after surgical interventions, the activity of antioxidant defense enzymes that characterize the condition of the skin, both intact and after damage, in the scar homogenate was studied. Skin samples were taken using a fine-needle biopsy. The samples were thoroughly cleaned of biological fluids by washing them with physiological solution and dried. Then, small pieces of material were homogenized. All parameters were recorded (homogenization time and revolutions per minute). Biological material (homogenates) of tissues were placed in Eppendorf tubes and centrifuged at 4000 rpm for 10 min. Tissue supernatants were used for further biochemical research. Such enzymes were superoxide dismutase and catalase, the activity of which in the forming scar tissue was determined on the 90th, 180th, and 360th days of observation of patients with different circadian rhythms who underwent planned surgical interventions in the maxillofacial localization in all studied groups [17], [18].

To determine the activity of superoxide dismutase, we used the method of Chevari *et al.* The principle of the method is based on the ability of the enzyme to inhibit the reduction of nitrotriazolium blue and to compete with nitroblue tetrazolium for superoxide anions, which are formed as a result of the aerobic interaction of the reduced form of nicotinamide adenine nucleotide with phenazine metasulfate [19].

Table 1: Clinical groups of patients

1 group 20 patients who were injected with a cryo extract of the placenta into the wound during the intraoperative period		2 group 20 patients who were injected with a cryo extract of the placenta into the wound during the intraoperative period in combination with electrophoresis of this drug on 90 th and 180 th day		3 group clinical (control) group 20 patients who received standard treatment	
1a with morning chronotype n = 10	1b with evening chronotype n = 10	2a with morning chronotype n = 10	2b with evening chronotype n = 10	3a with morning chronotype n = 10	3a with evening chronotype n = 10

Catalase activity in homogenates of scar tissue was determined according to the method of M.A. Koroliuk. The principle of the method is based on the ability of hydrogen peroxide to form a stable colored complex with ammonium molybdate, the intensity of which is inversely proportional to the activity of catalase in the investigated substrate. Catalase activity was studied by the spectrophotometric method by the amount of the colored product of reactions of hydrogen peroxide with ammonium molybdenum. For the experiment, tissue homogenate up to 2 ml was used, to which a 0.03% hydrogen peroxide solution was added, and then 0.1 ml of distilled water was added to the sample instead of the homogenate. The contents of both test tubes are incubated for 10 min. at a temperature of 37°C. The reaction is stopped by adding 1 ml of 4% ammonium molybdate. The contents of the test tubes are centrifuged for 10 min at 6,000 rpm. The intensity of the developed color is measured on a spectrophotometer at a wavelength of 410 nm against a control sample in which 2 ml of distilled water is added instead of hydrogen peroxide [20].

The observation period was used according to the literature data of epithelization and organization, which occurs up to 12 months [21]. The study was performed in accordance with the Helsinki Declaration on Ethical Principles for Medical Research involving Humans and approved by the Bioethics Commission of Poltava State Medical University (№211a, December 14, 2022).

Statistical analysis of obtained data was carried out by the parametric method using the Student's *t*-test.

Results

The activities of the antioxidant protection enzymes superoxide dismutase and catalase on 90th, 180th, and 360th day after surgical intervention are presented in Tables 2 and 3.

Analyzing the activity of the antioxidant protection enzyme superoxide dismutase in the homogenate of formed scar on the 90th day after the planned surgical intervention, it was established that in case of intrawound administration of cryoextract of the placenta on the background of standard treatment for patients with the morning chronotype (1a clinical group), its activity was significantly lower by 16.0% compared to the group of patients with the morning chronotype (3a clinical group) who were treated according to the

standard scheme for a similar period of observation (Table 2).

It should be noted that in the patients with the evening chronotype, there was no significant difference in the studied indicator relative to the 3b clinical group, which served as a control.

Table 2: Superoxide dismutase activity in the homogenate of the forming scar (U/mg)

	90 th day	180 th day	360 th day
1a clinical group n = 10	100.6 ± 2.88 (p ² < 0.05)	86.1 ± 2.41 (p ¹ < 0.05)	70.4 ± 2.14 (p ¹ < 0.05)
1b clinical group n = 10	106.8 ± 3.57	87.3 ± 2.55 (p ¹ < 0.05)	74.9 ± 2.16 (p ¹ < 0.05)
2a clinical group n = 10	94.5 ± 2.92 (p ² < 0.05)	81.3 ± 2.68 (p ¹ < 0.05) (p ² < 0.05)	66.3 ± 1.52 (p ¹ < 0.05) (p ² < 0.05)
2b clinical group n = 10	96.7 ± 2.56 (p ² < 0.05)	88.2 ± 2.05	67.9 ± 1.92 (p ¹ < 0.05)
3a clinical group n = 10	116.6 ± 4.09	95.4 ± 2.56 (p ¹ < 0.05)	75.4 ± 2.05 (p ¹ < 0.05)
3b clinical group n = 10	119.7 ± 3.81	97.1 ± 2.53 (p ¹ < 0.05)	76.3 ± 2.06 (p ¹ < 0.05)

n - the number of patients in the clinical group; p¹ - relative to the previous observation period within the same group; p² - relative to the control (3a and 3b clinical groups) for the same period of observation.

On the 180th day after surgical treatment, patients with the morning chronotype, who received intrawound injection of cryoextract of the placenta on the background of standard treatment, had a significant decrease of superoxide dismutase activity in the homogenate of the skin of the forming scar by 14.4% compared to the previous observation period, in the absence of significant changes relative to the 3a clinical group.

Table 3: Catalase activity in the homogenate of the forming scar (U/mg)

	90 th day	180 th day	360 th day
1a clinical group n = 10	65.3 ± 1.25 (p ² < 0.05)	57.1 ± 1.43 (p ¹ < 0.05)	53.9 ± 0.95
1b clinical group n = 10	66.4 ± 1.45 (p ² < 0.05)	58.2 ± 1.10 (p ¹ < 0.05)	55.3 ± 1.02
2a clinical group n = 10	63.1 ± 1.25 (p ² < 0.05)	55.7 ± 1.05 (p ² < 0.05) (p ¹ < 0.05)	52.7 ± 0.94
2b clinical group n = 10	65.5 ± 1.23 (p ² < 0.05)	56.5 ± 1.52 (p ¹ < 0.05)	53.5 ± 1.02
3a clinical group n = 10	78.6 ± 1.65	60.9 ± 1.52 (p ¹ < 0.05)	56.1 ± 1.06
3b clinical group n = 10	79.2 ± 1.50	61.2 ± 1.36 (p ¹ < 0.05)	56.5 ± 1.09

n - the number of patients in the clinical group; p¹ - relative to the previous observation period within the same group; p² - relative to the control (3a and 3b clinical groups) for the same period of observation.

Under the conditions of similar treatment for patients with an evening chronotype, a significant decrease in superoxide dismutase activity was 18.3%. In this clinical group, there was also no significant difference of the studied indicator relative to the control for a similar period of observation.

On the 360th day after surgery, a significant decrease in the activity of superoxide dismutase in the homogenate of the scar was noted in the indicated clinical groups by 18.2% and 14.2%, respectively, for the morning and evening chronotypes.

On the 90th day of observation in case of combined intrawound administration of placenta cryoextract and electrophoresis of this medicine, a significant decrease in the enzymatic activity of superoxide dismutase in the homogenate of the scar was noted relative to 3a and 3b clinical groups by 19.0% and 19.2%, respectively, in patients with morning and evening chronotypes.

At this stage of observation, no reliable differences of the superoxide dismutase activity were established in case of the same treatment among patients with different circadian rhythms.

At the next observation period, a significant decrease of superoxide dismutase activity by 14.0% was noted in the 2a clinical group of patients. This clinical group was also characterized by a significant decrease of the studied indicator by 14.8% compared to a group of patients with a similar circadian rhythm who were operated on using a standard treatment protocol.

For the 2b clinical group, changes in superoxide dismutase activity in a similar comparison were not reliable.

On the 360th day after surgical intervention in maxillofacial area during using of intrawound administration of placenta cryoextract in combination with electrophoresis of this drug, on the background of standard treatment, changes in the activity of the studied indicator were noted, which were similar to the results of patients in whom the administration of cryoextract of the placenta was not accompanied by its electrophoresis. Namely, a significant decrease in superoxide dismutase activity of the forming scars homogenate by 18.5% in patients with a morning chronotype and by 23.0% in patients with an evening chronotype. At the same time, in patients of the 2a clinical group, a significant decrease in superoxide dismutase activity was noted by 12.1% compared to the control (3a clinical group) for the same period of observation.

In case of using the standard treatment protocol, on the 90th day after planned surgical intervention in the maxillofacial area (3a and 3b clinical groups), no difference in the studied indicator was noted depending on the circadian rhythm of the patients, and this trend was observed at all periods of observation.

On the 180th day, a decrease in the activity of superoxide dismutase in the homogenate of the forming scar was noted, relative to the previous observation period, by 18.2% and 18.9%, respectively, for patients with the morning and evening chronotypes.

The tendency to decrease the activity of superoxide dismutase in the skin homogenate on the 360th day of the formation of scar tissue was preserved in control clinical groups, when treatment was carried out according to standard protocols, which was manifested by a reliable decrease in the activity of this indicator relative to the previous period of observation by 21.0% and 21.4%, respectively for patients with morning and evening chronotypes.

During the research, the activity of another important enzyme of antioxidant protection - catalase - was studied in the homogenate of scarred tissue on the same terms of observation.

It was established that on the 90th day after surgical intervention in patients with the morning chronotype who received intrawound injection of placenta cryoextract on the background of standard treatment, there was a significant decrease in catalase activity in the homogenate of the scar by 16.9% relative to 3a clinical group, in which patients had received standard treatment (Table 3).

During similar treatment of patients with an evening chronotype, a significant decrease in catalase activity relative to the control (3b clinical group) was 16.2%.

On the 180th day after the surgical intervention in the first clinical group, a significant decrease in catalase activity compared to the previous observation period was noted by 12.6% and 12.3% for the morning and evening chronotypes, respectively. At the same time, there were no significant changes in catalase activity relative to the control for a similar term of the post-operative period.

It should be noted that on the 360th day of observation, no significant difference in indicators was noted when comparing the results among patients of the morning and evening chronotypes in case of using the same treatment scheme and in comparison with the control. The tendency to decrease catalase activity in scar tissue was not statistically significant, regardless of the patients' circadian rhythm.

Under the conditions of additional application of electrophoresis of the placenta cryoextract, there were changes in the enzyme activity similar to the 1a and 1b clinical groups. Namely, a significant decrease in catalase activity by 19.7% and 17.3% for patients with morning and evening chronotypes, respectively. It was noted the absence of differences in the results depending on the circadian rhythm of the patients.

On the 360th day of observation, a tendency to decrease catalase activity continued to be registered in clinical groups 2a and 2b, but these changes were not reliable.

For patients who received treatment according to the standard protocol, on the 180th day after surgical intervention in the maxillofacial area, similar changes in the studied indicator were noted relative to the previous observation period. For patients with the morning chronotype, the decrease in catalase activity was 22.5%, with the evening –22.7%, in the absence of statistically significant changes in the enzymatic activity of catalase already on the 360th day of observation, which corresponded to the data of other clinical groups.

It was noted no significant difference in studied indicators in all clinical groups when comparing the

results among patients with the morning and evening chronotypes in case of using the same treatment regimen.

Discussion

Prevention of the formation of pathological scars is a significant medical and social problem. Thus, Zduńska *et al.* in their research describe that for better wound healing and for cosmetic purposes such as: Reduction of facial wrinkles, skin discoloration, they use antioxidant drugs, namely, ointments based on ferulic acid [22]. To solve this problem, we proposed the use of placenta cryoextract in the form of injection into the wound at the intraoperative stage, and with the help of electrophoresis at the postoperative stage.

To assess the formation of scarred tissue, we used such indicators of antioxidant protection as the activity of superoxide dismutase and catalase in the scar tissue homogenate. According to researchers, this is a complex system that not only takes part in providing energy to the entire body, but also supports human immunity, so Mu *et al.* in their research write the following, the stronger the antioxidant capacity of the human body, the healthier it will be [23]. A study by Oseguera-Castro *et al.* and other authors showed that a dietary intervention with antioxidant food supplements from spent coffee grounds as the main ingredient of cookies has a positive effect on the daily rhythm of a person, on improving sleep, and physical activity [24]. During the analysis of biochemical indicators of activity of antioxidant enzymes in the homogenate of the forming scar after surgical intervention in maxillofacial localization, we obtained the following data. An increase in the activity of superoxide dismutase and catalase, which was noted in all clinical groups, regardless of the type of treatment, daily rhythm. According to Hecker *et al.*, the authors in their studies demonstrated that the drug resveratrol has antioxidant properties and inhibits the formation of pathological scars. Thus, treatment with this drug already within a day helped to achieve an antiproliferative effect due to the induction of apoptosis, and the effect itself increased depending on the duration of the treatment, it was also noted that resveratrol suppressed the mRNA of procollagen type 1, which led to a decrease in the deposition of collagen in scarred tissue [25].

The activity of the antioxidant enzyme in the homogenate of the scar of maxillofacial localization, which is being formed, was characterized by a smaller dynamic of changes. A probable decrease in the activity of this enzyme relative to the previous observation period was noted only on the 180th day after surgery,

regardless of the type of treatment and daily rhythm. The decrease in the level of the studied indicator relative to control groups with morning and evening chronotypes occurred on the 90th day. Similar data were noted only in patients of the morning chronotype, who were intravenously administered cryoextract of the placenta in combination with electrophoresis. In the later period of observation, no significant changes in catalase enzymatic activity were observed in any of the studied groups. That supports the opinion of many researchers regarding the influence of circadian rhythms on the course of physiological and pathological processes in the body, namely, the relationship between human biological rhythms and the production of circadian hormones and their influence on the use of medicinal products. Vasko *et al.* and the authors investigated that there is a relationship between human biological rhythms and the production of circadian hormones. This study made it possible to reduce the dosage of the prescribed drugs and increase the effectiveness of the treatment in compliance with the principles of chronotherapy [26].

Taking into account the data of other researchers, we can state that the compliance of the time of surgical treatment with the patient's circadian rhythms and the use of cryoextract of the placenta as a preventive measure has a positive effect on the course of reparative regeneration of the skin after its damage.

Conclusions

Thus, according to our research, the enzymatic activity of catalase in the scar homogenate is characterized by a lower dynamics of changes than the activity of other enzyme of antioxidant protection - superoxide dismutase. Positive dynamics were noted under the conditions of use in the treatment protocol of placenta cryoextract, which was more pronounced in patients with a morning chronotype, especially in the earlier terms after surgical intervention in maxillofacial area.

The most significant changes were observed in the studied indicators of antioxidant protection in patients of the morning chronotype who underwent intrawound administration of cryoextract of the placenta in combination with electrophoresis of this drug against the background of a standard treatment protocol, especially at earlier terms after surgical treatment, which determines the favorable effect of this treatment on the processes reparative regeneration of the skin under the conditions of healing of the surgical wound in the maxillofacial area by primary tension.

Ethics Approval and Consent to Participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Poltava State Medical University, Poltava, Ukraine (protocol № 211a December 14, 2022).

Availability of Data and Materials

Data will be provided upon request.

Authors' Contributions

Conceptualization, Toropov OA; methodology Faustova MO; validation Lokes KP; formal analysis, Toropov OA., Steblovskyi DV., and Bukhanchenko OP.; investigation, Topov OA., Faustova MO.; resources, Toropov OA., Steblovskyi DV; data curation, Bukhanchenko OP.; writing—original draft preparation, Toropov OA. and Yatsenko IV.; writing-review and editing, Toropov OA., Avetikov DS; supervision, Avetikov DS., and Faustova MO.; project administration, Avetikov DS and Lokes KP. All authors have read and agreed to the published version of the manuscript.

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