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Original article

DYNAMIC CHANGES OF TISSUE ENZYMES AND THE ITS INFLUENCE ON WOUND HEALING OF NASAL MUCOSA IN THE POSTOPERATIVE PERIOD

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ABSTRACT

Intranasal surgery is widely spread all over the world and has the leading position among all otorhinolaryngological surgical interventions. However, any surgery makes sensitive trauma to the ciliary epithelium that covers the nasal cavity and paranasal sinuses despite the modern technics of operations. That's why reveling peculiarities, patterns and ways of correction of nasal mucosa regeneration in the postoperative period is very important for clinicians. So the main aim of our paper is to investigate the role of some tissue enzymes in the process of nasal mucosa regeneration after surgical interventions on the structures of the nasal cavity in adults.

As a result of our investigation, the role of some tissue enzymes was shown. We have based the usage of drugs that influence wound healing.

Thus, the proper medicines have positive effects on the regeneration processes of the ciliated epithelium. A more thorough investigation of the role of these enzymes will allow developing methods of its correction for improving the efficiency of endonasal surgery rapid and qualitative recovery of mucociliary clearance.

Keywords: ciliated epithelium, intranasal surgery, transforming growth factor-beta, matrix metalloproteinases,

INTRODUCTION

As we know, healthy airway surfaces are lined by ciliated epithelial cells and covered with the layer, which has two components: a mucus layer that entraps inhaled particles and foreign pathogens and a low viscosity periciliary layer that lubricates airway surfaces and facilitates ciliary beating for efficient mucus clearance. The only coordinated function of these components provides correct and effective mucociliary clearance – the main protection of airways from different pathological influences [1, 2].

Statistically, nasal septum deviation is one of the most common endonasal pathology that leads to a various spectrum of problems: starting from subjective complaints of the patient and ending with disturbances of mucociliary clearance, morphological changes of the nasal mucus layer and progressing of comorbid pathology. Obviously, there is only a surgical method for the correction of these conditions [3,4].

Even using endoscopies technics during surgical interventions does not allow to avoid trauma of the nasal mucosa. There is no doubt that the postoperative trauma of nasal mucosa may lead to different complications as in early postoperative period and in the distant period [5, 6, 7]. Moreover, nasal packing, which is very common after nasal surgery, also causes additional trauma to the mucosa.

The detailed sequence of wound healing of the nasal respiratory mucosa is delicately regulated. The aim of wound healing is a structural and functional repair of the tissue defect. Preservation of the basal membrane results in rapid restoration of normal epithelial height, while its destruction will lead to a repair process taking weeks to months. Deeper wounds will also result in scar formation, with its extent being quite variable [8]. Cytokines and growth factors regulate wound healing of the mucosal lining through the recruitment of inflammatory leukocytes and stimulation of fibroblasts and epithelial cells [9].

Unfortunately, the methods of correction, acceleration of regeneration and prevention of scar formation of nasal mucosa after intranasal surgery are only being studied. Thus, we decided to investigate the role of some enzymes on the wound healing process and the influence of some substances on to speed and quality of regeneration.

Numerous biological active substances take part in the processes of mucosa regeneration. Among them, transforming growth factors and matrix metalloproteinases play a key role in all phases of this process [10, 11].

In our work, we tried to find a correlation between the levels of transforming growth factor beta 1(TGF-b1) and a member of the matrix metalloproteinase group - matrix metalloproteinase 9 (MMP-9)– with the quality and fullness of nasal mucosa regeneration after surgical correction of nasal septum deviation and hypertrophy of inferior nasal turbinates. Besides, we also investigated the influence of some exogenous substances on these processes.

MATERIAL AND METHODS

Under observation, there were 78 persons, which formed three groups. Each group had 26 persons. The structures of groups were approximately similar and consisted of men and women of age 18-50 years with the diagnosis "Deviated nasal septum and hypertrophy of inferior nasal turbinates" without verified systemic pathology and allergy. All these patients were undergone surgical correction of nasal septum deviation (septoplasty) and plastic of inferior turbinates. The technic of operation was the same in all cases. In the postoperative period, patients received vacuum aspiration of nasal secrets and removing of big hemorrhagic crusts from the nasal cavity under local anesthesia.

The first group additionally obtained isotonic solution in spray form, and after 30 minutes' nasal drops contained the substance deoxyribonucleate sodium. The second group used only isotonic solution spray, the third group received only mechanical cleaning of the nasal cavity. As the third group did not receive any additional treatment, we pointed it as a control. Treatment in the postoperative period started from the first day after surgery.

TGF-β1 and MMP-9 were detected in nasal mucus by enzyme-linked immunosorbent assay on the 1, 2, 3 and 4 weeks according to phases of wound healing. Nasal mucus was obtained from the nasal cavity in accordance with the modified method proposed by Jean-Baptiste Wateletet al. [12].

TGF-β1 is secreted only in latent form and is activated commonly after damaging tissues [13]. Also, MMP, including MMP-9, are secreted in non-active forms in case of absence tissue trauma.

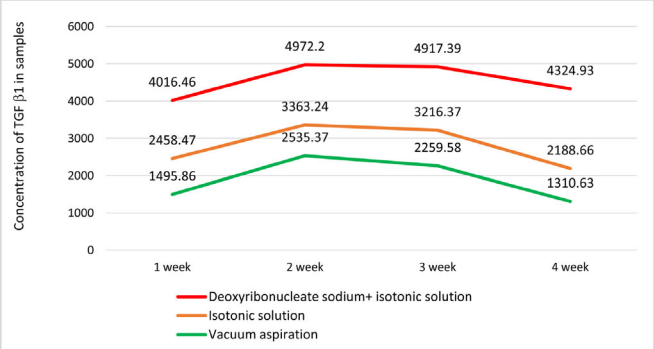
For visual assessment of nasal mucosa healing, we used daily endoscopic examination. We paid attention to the severity of edema, crusts formation, and integrity of nasal mucosa. For the first group of patients, we chose drops with deoxyribonucleate sodium because of their proven positive influence on regeneration processes in different areas of medicine. However, its role in wound healing after intranasal surgery is still not investigated [14, 15].

Statistically, we used the Shapiro-Wilk W test as a test of normality. We established the number of W criteria and the level of statistical significance correlated with it. Pe"0,05 meant that distribution was not differed from normal and could be used for checking of statistical hypothesis.

RESULTS

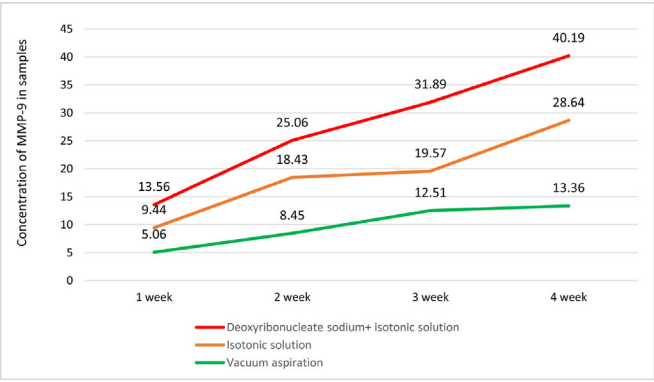
Patients that received deoxyribonucleate sodium drops and isotonic solution spray had a higher level of TGF-β1 than the patients from the other two groups on the first week of postoperative. All groups showed the peak content of this substance on the second week with a gradual decrease up to the fourth week. Nevertheless, the concentration of this enzyme stood higher on the fourth week in the first group compared with the other two groups (Figure 1).

Fig. 1. Dynamic of changes of TGF-β1 in samples



MMP-9 showed an obviously ascending type of curve. We detected a constant rising of this enzyme in all groups of the patients, beginning from the first week. The maximal levels of MMP-9 we received in the first group of the patients (Figure 2).

Fig. 2. Dynamic of changes of MMP-9 in samples



During the endoscopic examination, we revealed more intensive and rapid processes of regeneration in the first group of patients. It manifested in less formation of crusts, more intense resolution of postoperative edema and faster restoration of nasal breathing. In most patients of this group, nasal mucosa looked almost normally by the 10-day postoperative period and also, these patients had fewer subjective complaints. The main signs and the progress of symptoms resolution in patients of the first group are shown in table 1.

Table 1. Dynamic of nasal symptoms in a patient of the first group

Symptoms	Period of examination, weeks	Number of patients, %
Improving of nasal breathing	1	60
	2	85
	3	100
	4	100
Reducing of	1	50
	2	75

mucosa edema	3	100
	4	100
Reducing in crusts formation	1	65
	2	80
	3	100
	4	100

The processes of wound healing in the other two groups were slower, and it took on average 12 days in the second group (22 persons that correspond 84%) and 14-15 days in the third group (20 persons that correspond 77%). We noticed the prolonged formation of crusts and dystrophy of mucosa under the crusts, especially in the third group (11 patients that correspond 42%). Absolute numbers of enzymes changes are shown in table 2.

Table 2. Absolute numbers of enzymes during the period of observing

Group	Substance	1 week (M±m)	2 week (M±m)	3 week (M±m)	4 week (M±m)
Deoxyribonucleate sodium+ isotonic solution (n=28)	MMP-9	13,56±0,72 W=0,946, p≥0,1	25,06±1,66 W=0,984, p≥0,1	31,89±1,48 W=0,941, p≥0,1	40,19±1,98 W=0,966, p≥0,1
	TGF	4016,46±44,02 W=0,959, p≥0,1	4972,20±42,91 W=0,945, p≥0,1	4917,39±43,92 W=0,949, p≥0,1	4324,93±35,96 W=0,958, p≥0,1
Isotonic solution (n=28)	MMP-9	9,44±0,63 W=0,975, p≥0,1	18,43±1,14 W=0,980, p≥0,1	19,57±1,42 W=0,959, p≥0,1	28,64±1,64 W=0,953, p≥0,1
	TGF	2458,47±26,26 W=0,953, p≥0,1	3363,24±34,48 W=0,956, p≥0,1	3216,37±35,03 W=0,940, p≥0,1	2188,66±32,02 W=0,943, p≥0,1
Vacuum aspiration (n=28)	MMP-9	5,06±0,46 W=0,962, p≥0,1	8,45±0,65 W=0,973, p≥0,1	12,51±0,96 W=0,985, p≥0,1	13,36±1,10 W=0,974, p≥0,1
	TGF	1495,86±19,82 W=0,947, p≥0,1	2535,37±28,16 W=0,968, p≥0,1	2259,58±29,11 W=0,934, p≥0,1	1310,63±25,78 W=0,966, p≥0,1

DISCUSSION

The effect of TGF-β1 on reepithelialization appears paradoxical; its expression by keratinocytes after wounding together with the inhibitory effect of TGF-b on keratinocyte proliferation in vitro and in vivo suggests TGF-b as a negative regulator of reepithelialization. On the other hand, it also induces the expression of integrins necessary for keratinocyte migration across the fibronectin-rich provisional wound matrix, and exogenous TGF-b was shown to stimulate keratinocyte migration and wound reepithelialization [16, 17].

An experimental animal model of the wound showed that TGF-β1 is objectively able to accelerate healing in vivo [18]. In other works, local applications of TGF-β1 led to faster closing of tympanic membrane perforation in rats. Besides, the positive role of TGF-β1 was demonstrated during the healing of chronic ulcers and keloids in humans and stimulation of intestinal mucosa in mice [17]. On the other hand, it is detected that patients with chronic rhynosinusitis have higher levels of TGF-β1 in the extracellular matrix, and it may lead to the development of fibrosis areas [19].

Matrix metalloproteinases, including MMP-9, are the other big group of proteinases involved in regeneration processes. On the one hand, these enzymes are investigated as markers of fibrosis in some chronic processes, including chronic rhinosinusitis and nasal polyposis [10]. On the other hand, there is evidence of the positive role of MMP-9 on reepithelization [21, 22].

In our investigation, higher levels of TGF-β1 and MMP-9 in the postoperative period correlated with better and faster wound healing. This fact also was proved with daily endoscopic revision of the nasal cavity.

We can propose a few explanations for such results. Firstly, we support the position of Balsalobre et al. that the activity and, probably, the role of some enzymes, for example, TGF-β1, may differ in different layers of mucosa. They noticed that level of this enzyme in some chronic pathology is lower in epithelial cells but increased in stroma compared with healthy mucosa [23]. On the other hand, the influence and activity of MMP-9 and TGF-β1 may depend on the background of the pathological process. It is possible that in the case of chronic inflammation, activity and actions of investigated enzymes may be

changed and lead to such pathological type of regeneration as scar tissue formation. As the activity of MMP-9 and TGF-β1 is not similar in different layers of mucosa, we also may make a conclusion that surgical trauma of nasal mucosa must be minimal to prevent the excess secretion of these enzymes.

CONCLUSIONS

Wound healing of nasal mucosa after intranasal interventions is the second key point in rhinology after the surgery itself. The final result strongly depends on the way the wound healing will pass. Only complete restoration of morphology and physiology of mucociliary transport prevents complications and bad surgery outcomes. That's why it is necessary to seek the possibility to improve and accelerate the regeneration of nasal epithelium after its damage. But as it was shown in our work, the role of some enzymes in wound healing is controversial, and the number of factors may influence its activity as well, even on the final result of its functioning. In fact, this area of otorhinolaryngology and adjacent disciplines requires continuous investigations.

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