

RESEARCH OF MITOMYCIN-C SATURATION IN DACRYOCYSTORHINOSTOMY OSTEOTOMY SITE TISSUE

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✧ **Introduction.** Mitomycin-C is an alkylating antibiotic used to prevent excessive scar formation in the dacryostoma area after dacryocystorhinostomy. *In vitro* studies proved its inhibition effect on fibroblast growth in 0.2 mg/ml concentration. Up to date, clinical data on its efficacy remain contradictory. **Aim.** To evaluate the concentration of Mitomycin-C in nasal cavity and lacrimal sac mucosa after topical application and to determine the clinical efficacy of this procedure. **Materials and methods.** 30 patients with nasolacrimal duct obliteration underwent an endonasal endoscopic dacryocystorhinostomy. At the end of the surgery, at the osteotomy site, a sponge soaked in Mitomycin-C 0.2 mg/ml was applied for 3 minutes. Nasal mucosa biopsy was performed immediately after application, in 30 minutes, and at the 1st day after surgery. Biopsy material analysis of was performed by liquid chromatography – mass spectrometry. The surgical treatment efficacy was established according to proposed efficacy criteria. **Results.** The analysis of Mitomycin-C concentrations established them to be: 626 ± 176 ng/g tissue immediately after application, 230 ± 61 ng/g tissue in 30 minutes after application. In 24 hours after surgery, there was no Mitomycin-C in the tissue. Surgical efficacy was 86.7%, recurrences were found in 13.3% of cases. **Conclusion.** Surgery clinical results coincide with those obtained by other researchers. But chemical investigation showed that Mitomycin-C tissue concentration was lower than that in previous *in vitro* studies.

✧ **Keywords:** nasolacrimal duct; endoscopic endonasal dacryocystorhinostomy; Mitomycin-C; dacryocystitis.

ИЗУЧЕНИЕ НАСЫЩЕНИЯ ТКАНЕЙ ДАКРИОСТОМЫ МИТОМИЦИНОМ-С

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✧ **Введение.** Для профилактики избыточного рубцевания области дакриостомы после дакриоцисториностомии используют алкилирующий антибиотик Митомин-С. В исследованиях *in vitro* было доказано его подавляющее действие на образование фибробластов в концентрации 0,2 мг/мл. Однако клинические данные об его эффективности до настоящего времени остаются противоречивыми. **Цель исследования:** определить концентрацию Митомин-С в слизистой оболочке полости носа и слёзного мешка при аппликационном введении и оценить его клиническую эффективность. **Материал и методы.** 30 пациентам с облитерацией слезоотводящих путей на уровне шейки слёзного мешка была выполнена эндоназальная эндоскопическая дакриоцисториностомия. На заключительном этапе в область сформированной дакриостомы помещали турунду с Митомин-С в концентрации 0,2 мг/мл на 3 минуты. Биопсии слизистой оболочки полости носа и слёзного мешка осуществляли сразу после применения препарата, через 30 минут, на 1-е сутки после операции. Анализ полученных образцов осуществляли при помощи высокоэффективной жидкостной хроматографии — масс-спектрометрии. Оценку эффективности хирургиче-

ского вмешательства проводили по предложенным ранее критериям. **Результаты.** По данным анализа концентрация Митомоцина-С сразу после введения составила 626 ± 176 нг на 1 г ткани, через 30 минут — 230 ± 61 нг на 1 г ткани, через сутки после операции препарат обнаружен не был. В результате операции положительные результаты составили 86,7 % случаев, «рецидивы» — 13,3 % случаев. **Выводы.** Клинические результаты проведённого хирургического лечения совпадают с результатами, полученными другими исследователями. Однако химическое исследование показало, что концентрация препарата в тканях значительно ниже, чем заявленная концентрация в исследованиях *in vitro*.

✧ **Ключевые слова:** слезоотводящие пути; эндоназальная эндоскопическая дакриоцисториностомия; Митомоцин-С; дакриоцистит.

BACKGROUND

Mitomycin C is an alkylating antibiotic produced by *Streptomyces caespitosus*. It inhibits cellular RNA, DNA, and protein synthesis; in particular, it suppresses collagen production by fibroblasts, which reduces fibrotization in the area of surgical intervention [1, 2]. Mitomycin C is used for the prevention of excessive scarring after dacryocystorhinostomy, and was first used by Urugba et al. in 1997. Mitomycin C (0.5 mg/mL) was topically applied in the dacryostoma area for 2.5 min with subsequent evaluation of its efficacy by morphological examination. The local application of mitomycin C induced intercellular edema and changes in the mitochondria and the cell nucleus. It was assumed that mitomycin C potentially improves the effectiveness of dacryocystorhinostomy [3]. Thereafter, many researchers started exploring the intraoperative applications of the antibiotic, trying various concentrations (0.2–0.5 mg/mL) [4, 5] and expositions (2–10 min) [6, 7]. The efficacies of these surgeries varied between 78.5% and 100% [8].

In the Russian Federation, mitomycin C was first studied by Beloglazov et al. A turunda with the antibiotic (0.5 mg/mL) was applied in the dacryostoma area for 5 min at the final stage of endonasal dacryocystorhinostomy according to the procedure protocol originally suggested by West and modified by Beloglazov. Patients were followed up for 6 months. The rates of successful outcomes were 93.2% and 72.5% in the experimental and control groups, respectively; thus, the efficacy of mitomycin C was confirmed clinically [9].

A number of studies have proved the effectiveness of mitomycin C in repeated surgeries [10]; other authors combined the application of mitomycin C with the intubation of dacryostoma with a silicone implant [7, 11]. However, the existing disparity in the results of multiple studies and the lack of proper clinical trials prevent us from making

an unambiguous conclusion about the role of mitomycin C in the prevention of excessive scarring and associating it with a recurrence of lacrimal ducts obliteration.

In 2013, Ali et al. used different concentrations of mitomycin C to explore its cytopathic effect on the culture of human nasal mucosal fibroblasts and found that the optimal concentration of mitomycin C ensuring the best antifibrotic activity is 0.2–0.3 mg/mL [12]. Using electron microscopy, they also evaluated ultrastructural effects of topical Mitomycin C (0.2 mg/mL, 3-min exposition) on nasal mucosa after endonasal dacryocystorhinostomy. Topical Mitomycin C was demonstrated to induce ultrastructural changes in the epithelium, vessels, and fibroblasts [13].

Therefore, a significant amount of data on the use of mitomycin C as an antifibrotic agent has been accumulated so far.

The existing disagreement on the clinical efficiency of mitomycin C is probably caused by the insufficient concentration of the drug in the tissues surrounding dacryostoma; however, this aspect has never been explored till date. Modern methods of chemical analysis enable the detection of extremely low concentrations of an analyte in biological specimens. Pharmacokinetics studies suggest the evaluation of changes in the concentration of the drug over time, which requires multiple specimens collected at different time periods. The analysis implies the assessment of an isolated tissue sample of a standard weight containing the analyte.

High-performance liquid chromatography–mass spectrometry (HPLC–MS) is an extremely sensitive method that allows the analysis of microscopic specimens, which ensures maximum safety for a patient.

This study was designed to assess the tissue concentration of mitomycin C in the dacryostoma area after its topical application during the endoscopic endonasal dacryocystorhinostomy.

MATERIALS AND METHODS

In this monocenter, randomized, open-label, prospective study, 30 patients with obliteration of the lacrimal duct at the level of the lacrimal sac were enrolled. The study population included 4 males and 26 females, aged 63 ± 15 years. This study was approved by a local ethics committee. Informed consent was obtained from each patient after providing all necessary clarifications regarding the study.

We did not include patients with traumatic injuries to the lacrimal ducts and their secondary changes, those who earlier underwent surgical treatment of the lacrimal ducts, and those with any diseases of the nasal cavity and paranasal sinuses requiring treatment. We performed standard ophthalmic and dacriologic examinations of the study participants. Tearing symptoms were evaluated using the Munk's scoring system. All patients underwent lacrimal meniscometry using optical coherence tomography, nasal endoscopy, and contrast-enhanced multislice computed tomography of the lacrimal ducts.

Endoscopic endonasal dacryocystorhinostomy was performed according to the method by P. Wormald with several modifications: during dacryostoma formation, the medial wall of the lacrimal sac was dissected along the perimeter of the bone window, and the fragment of the nasal mucosa was resected up to the level of the posterior side of dacryostoma. At the final stage of the surgery, a turunda soaked in 2 mL of Mitomycin C (0.2 mg/mL) was inserted into the dacryostoma area for 3 min. After its removal, the lacrimal ducts were rinsed with saline solution. All patients underwent optically controlled biopsy of the nasal mucosa and lacrimal sac (Karl Storz endoscope, 3.4-mm diameter, 30 scope). Second and third biopsy were performed at 30 min and at 24 h post-surgery, respectively.

Before the analysis, specimens were placed in 1-mL deionized water, weighted using precision scales (Sartorius AG, Germany) with accuracy up to 0.001 g, and incubated in an ultrasonic bath (Daihan Scientific, Korea) for 15 min. Samples were stored at 5°C for no more than 24 h. We determined the concentration of mitomycin C in the solution obtained, then adjusted it by the weight of the tissue sample, and calculated the concentration of Mitomycin C in the original specimen.

The concentration of mitomycin C was measured by HPLC–MS using the Agilent 1260 liquid chromatograph (Agilent, USA) with the maXis Impact Mass Spectrometric Detector (Bruker, Germany)

with electrospray ionization, quadrupole, and time-of-flight mass analyzers (Figure 1).

Separation was performed using the Zorbax SB-C18, 150 mm × 4.6 mm × 5.0 m column (Agilent); isocratic elution mode, 100% acetonitrile (JT Baker, The Netherlands); and speed of the mobile phase, 0.25 mL/min. The total time of the analysis was 2.1 min.

We compared the clinical outcomes of 30 study participants in whom surgical interventions were followed by the application of Mitomycin C with the outcomes of 30 randomly selected patients who underwent similar surgical interventions without Mitomycin C application in the same clinic between January and November 2015.

The treatment results were monitored on a weekly basis during the first month after surgery and then at 3 and 6 months post-surgery. The final examination included the evaluation of tearing symptoms using the Munk's scoring system, dye disappearance test, assessment of the lacrimal meniscus height using optical coherence tomography, assessment of the permeability of the lacrimal ducts, and endoscopy of the nasal cavity and the dacryostoma area. The treatment outcomes were classified as follows:



Fig. 1. Mass spectrometer detector Maxis Impact (Bruker, Germany)

Рис. 1. Масс-спектрометрический детектор Maxis Impact (Bruker, Германия)

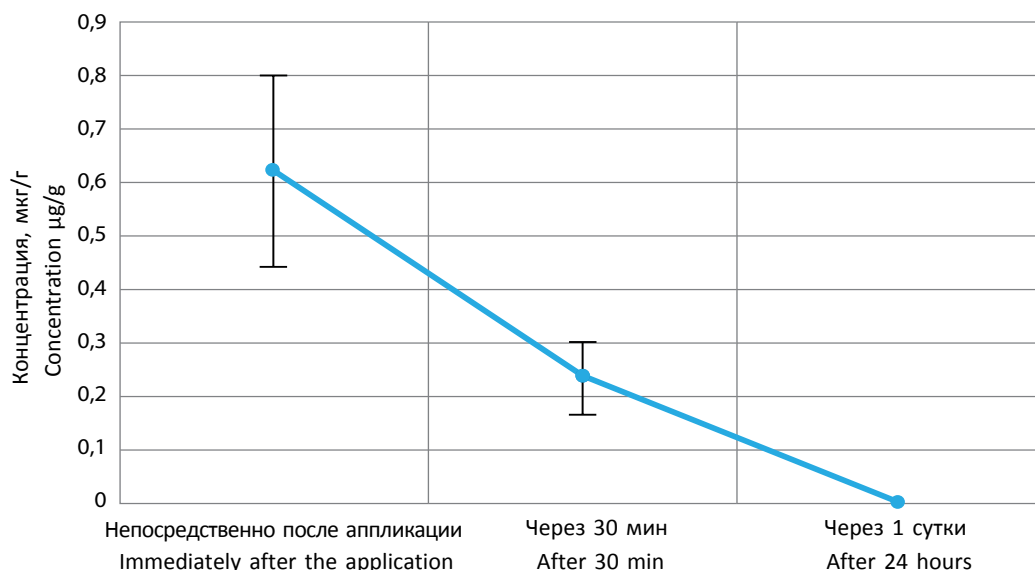


Fig. 2. Dependence of Mitomycin-C mcg/g concentration on time

Рис. 2. Зависимость концентрации Митомицина-С, мкг/г, от времени

“cured,” “improvement” (successful outcome), and “relapse” (unsuccessful outcome) [14].

The data analysis was performed using MS Excel 2007, and we calculated mean values and standard deviations.

RESULTS AND DISCUSSION

We observed that the tissue concentration of Mitomycin C was 0.626 ± 0.176 µg/mL immediately after the application, and 0.23 ± 0.06 µg/mL 30 min after the application. No Mitomycin C was detected in the biopsy material collected 24 h after injection (Figure 2). The results of surgical treatment are shown in Table 1.

Upon final examination at 6 months post-surgery, the epithelial lining of the dacryostoma area was observed in 26 patients, whereas 6 participants had scar tissue covering the dacryostoma area. A total of 86.7% of patients had successful outcomes, of whom 66.7% were considered cured and 20.0% showed improvement. Relapses were registered in 13.3% of patients.

Surgery clinical results

Клинические результаты хирургического лечения

Period	Intensity of tearing according to Munk, points	Dye disappearance test (nasal test), cases (%)		Lacrimal meniscus height, mm
		Positive	Negative	
Before surgery	3.8 ± 0,8	0 (0 %)	30 (100 %)	0,67 ± 0,13
		26 (86.7 %)	4 (13.3 %)	
After surgery	1.1 ± 0,3	0 (0 %)	30 (100 %)	0,26 ± 0,09
		26 (86.7 %)	4 (13.3 %)	

CONCLUSION

The results of our study demonstrate that the concentration of Mitomycin C in tissues surrounding dacryostoma immediately after its application is 0.626 ± 0.176 µg/mL, which is approximately 300 times lower than the concentration proven to demonstrate an effective antifibrotic effect in vitro (0.2–0.3 mg/mL). Therefore, the lack of convincing data on the efficacy of mitomycin C is likely to be due to insufficient tissue concentrations of the drug after its topical application.

Because earlier studies have already proved the ability of Mitomycin C to inhibit the activity of fibroblasts, further research should be focused on the development of measures that will allow increasing Mitomycin C concentrations in tissues surrounding dacryostoma.

The authors declare no conflict of interest.

Authors' contribution:

Development of a research concept and study design – E.L. At’kova, A.O. Root, and S.D. Yartsev.

Table 1

Таблица 1

Data collection and processing — A.O. Root, N.N. Krakhovetskiy, S.D. Yartsev, and V.D. Yartsev
 Data analysis and drafting the manuscript—E.L. Atkova, A.O. Root, and S.D. Yartsev

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