Original Research

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Assessment of the microbiological picture of the apical granuloma content against the background of treatment with the stromal-vascular fraction of adipose tissue and an osteogenesis stimulator in the experiment

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Abstract

INTRODUCTION. Patients with various forms of periodontitis account for 30–40% of all clinic visitors. Among chronic forms of periodontitis, destructive forms predominate. Among those seeking emergency dental care, patients with acute or exacerbated chronic periodontitis account for 75%. Long-term inflammation of the periodontal tissues is the most common cause of tooth loss and the formation of odontogenic infection foci. The presence of an infection foci poses a risk due to the progressive increase in pathogens and bacteriotoxins in the oral fluid. The quality of treatment outcomes depends on the medicamental and instrumental treatment of the root canals and the complete obturation of the root canal system.

AIM. To study the microflora of the apical granuloma and develop a treatment protocol.

MATERIALS AND METHODS. The study was conducted on 83 rabbits. Clinical, radiographic, and microbiological methods were used in the experiments. The animals were divided into two groups: the main group ($n = 43$) and the control group ($n = 40$). The main group received treatment according to the protocol developed by the authors, using a stromal-vascular fraction in combination with an osteogenesis stimulator. The control group received standard therapy, which included endodontic root canal treatment followed by extraapical filling.

RESULTS. Before treatment, high levels of microbial contamination of the apical granuloma were detected in the animals: Gram-positive cocci were 2.8×10^7 CFU/ml, Gram-negative cocci – 1.9×10^7 CFU/ml. After treatment, the main group demonstrated a decrease in the number of Gram-positive and Gram-negative cocci by 0.7×10^6 CFU/ml ($p < 0.001$). Six and 12 months after therapy, the results remained stable, and a further decrease in the number of pathogenic microorganisms was noted. In the control group, the titers of microbial contamination of the apical granuloma contents decreased by 0.2×10^5 ($p < 0.001$) after treatment. However, after 6 months, microbial contamination returned to baseline levels. Twelve months after treatment, an increase in contamination by 0.3×10^6 CFU/ml was observed.

CONCLUSION. An analysis of the results of treatment for apical destructive forms of periodontitis using a stromal-vascular fraction and an osteogenesis stimulator under experimental conditions demonstrated high therapeutic efficacy in 99.9% of cases. Furthermore, an evaluation of the treatment protocol proposed by the authors confirmed a reduced incidence of complications and a stable therapeutic effect over a long-term observation period.

Keywords: stromal-vascular fraction, root canal, apical periodontitis, apical granuloma, microflora, osteogenesis stimulator, animals, experiment

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Оценка микробиологической картины содержимого апикальной гранулемы на фоне лечения стромально-васкулярной фракцией жировой ткани и стимулятором остеогенеза в эксперименте

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Резюме

ВВЕДЕНИЕ. Пациенты с различными формами периодонтита составляют 30–40% от общего числа обратившихся в клинику. Среди хронических форм периодонтита преобладают деструктивные формы. Среди обратившихся за неотложной стоматологической помощью, пациенты с острым или обострившимся хроническим периодонтитом составляют 75%. Длительное воспаление тканей периодонта является наиболее частой причиной потери зубов и формирования очагов одонтогенной инфекции. Наличие очага инфекции представляет опасность за счет прогрессирующего увеличения патогенов и бактериотоксинов в ротовой жидкости. Качество результатов лечения зависит от медикаментозной, инструментальной обработки каналов зубов и полноценной обтурации системы корневых каналов.

ЦЕЛЬ. Изучить микрофлору содержимого апикальной гранулемы и разработать протокол лечения.

МАТЕРИАЛЫ И МЕТОДЫ. Исследование проводилось на животных – кроликах (n = 83). В экспериментах применялись клинические, рентгенологические и микробиологические методы. Животные были разделены на две группы: основную (n = 43) и контрольную (n = 40). Основная группа получала лечение, по разработанному авторами протоколу, с использованием стромально-сосудистой фракции в сочетании со стимулятором остеогенеза. Контрольная группа получала стандартную терапию, которая включала эндодонтическую обработку корневых каналов с последующим экстраапикальным пломбированием.

РЕЗУЛЬТАТЫ. До начала лечения у животных были обнаружены высокие уровни микробного загрязнения содержимого апикальной гранулемы: грамположительные кокки составляли $2{,}8 \times 10^7$ КОЕ/мл, грамотрицательные кокки – $1{,}9 \times 10^7$ КОЕ/мл. После лечения в основной группе наблюдалось снижение количества грамположительных и грамотрицательных кокков на $0{,}7 \times 10^6$ КОЕ/мл ($p < 0{,}001$). Через 6 и 12 месяцев после терапии результаты оставались стабильными и отмечалось дальнейшее снижение количества патогенных микроорганизмов. В контрольной группе титры микробного загрязнения содержимого апикальной гранулемы после лечения снизились на $0{,}2 \times 10^5$ ($p < 0{,}001$). Однако через 6 месяцев микробное загрязнение вернулось к исходному уровню. Через 12 месяцев после лечения наблюдалось увеличение загрязнения на $0{,}3 \times 10^6$ КОЕ/мл.

ЗАКЛЮЧЕНИЕ. Анализ результатов лечения апикальных деструктивных форм периодонтита с использованием стромально-сосудистой фракции и стимулятора остеогенеза в экспериментальных условиях показал высокую терапевтическую эффективность в 99,9% случаев. Кроме того, оценка результатов, предложенного авторами протокола лечения, подтвердила снижение частоты осложнений и стабильный терапевтический эффект в течение длительного периода наблюдения.

Ключевые слова: стромально-васкулярная фракция, корневой канал, апикальный периодонтит, апикальная гранулема, микрофлора, стимулятор остеогенеза, животные, эксперимент

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INTRODUCTION

Improving the quality of treatment for patients with periodontitis remains a pressing clinical challenge. The primary cause of unfavorable outcomes in the management of teeth with chronic apical periodontitis is inadequate debridement and disinfection of the root canal system [1–3].

The diversity of therapeutic approaches to chronic apical periodontitis contributes to variability in clinical decision-making among dental practitioners. These strategies are aimed at eliminating exudative and destructive inflammation in the periapical tissues, achieving detoxification, stabilizing immune mechanisms, and preventing disease recurrence. Accordingly, there is a clear need for comparative evaluation of existing treatment modalities, as well as for continued scientific efforts to refine established methods and to develop novel comprehensive therapeutic approaches for chronic apical periodontitis [4–6].

Stem cells were first described by the Russian scientist, histologist, and professor, head of the Department of Histology and Embryology at Petrograd University, and founder of the unified concept of hematopoiesis, A.A. Maximov [7]. In 1891, the renowned French scientists Charles-Édouard Brown-Séquard (physiologist and neurologist) and Jacques-Arsène d'Arsonval (physiologist, physicist, and pioneer of biophysics) attempted to treat a patient with leukemia through oral administration of bone marrow extract, anticipating its hematopoietic therapeutic effect. This represented the first attempt to utilize bone marrow-derived stem cells, however, the experiment was unsuccessful.

Stem cells facilitate the regeneration of various tissue defects by activating the intrinsic reparative capacity of the organism, thereby promoting the restoration of damaged organs and tissues. At present, particular attention is focused on the ability of stromal stem cells to differentiate into osteogenic lineages, which is of significant relevance for bone tissue regeneration [3; 8–11].

A current priority in modern dentistry is the experimental and clinical substantiation of the efficacy of porous osteoconductive materials enriched with the stromal vascular fraction of adipose tissue for the reconstruction of alveolar ridge defects in the management of destructive processes.

AIM

The aim of this study was to normalize the microflora of the apical granuloma contents in periodontitis through the experimental application of the stromal vascular fraction and an osteogenesis stimulator.

MATERIALS AND METHODS

Clinical, radiological (periapical radiovisiography), and microbiological methods were employed in the experimental animals.

A total of 83 animals with experimentally induced apical periodontitis were treated using the stromal vascular fraction in combination with an osteogenesis stimulator. The stromal vascular fraction was isolated from autologous adipose tissue.

The biomaterial Alloplant is a decellularized tissue matrix characterized by a defined fibroarchitectonics, mechanical properties, and histochemical composition, and is processed by radiation sterilization and laser modeling (Registration Certificate No. FSR 2011/12012, dated February 3, 2015).

Initially, experimental periodontitis was induced in the animals, followed by microbiological analysis of the apical granuloma contents. Microbiological assessment included determination of the total microbial load, represented by associations of Gram-positive and Gram-negative microorganisms, enabling evaluation of the nature of the purulent-inflammatory process and the effectiveness of the treatment. Sampling was performed through the root canal using sterile paper points and a sterile instrument, with subsequent placement into AMIES transport medium (APEXLAB). To quantify microbial content in the studied biotope, 0.1 mL of microbial suspension from serial dilutions was inoculated onto culture media, including 5% blood agar and Endo agar.

Intergroup comparisons of mean values were performed using Student's *t*-test. All parameters were recorded at baseline (day 1), at the completion of treatment, and at 6- and 12-month follow-up intervals.

Following radiological assessment, the animals with periapical periodontitis without fistula were allocated into two groups: the main group ($n = 43$) and the control group ($n = 40$).

The main group received the treatment protocol developed by the authors. All procedures were performed under ether anesthesia. After endodontic preparation, the stromal vascular fraction and the osteogenesis stimulator were combined and delivered into the apical granuloma and the root canal of the central incisors of rabbits using a lentulo spiral. Prior to application, the osteogenesis stimulator was preconditioned in 0.9% sodium chloride solution at a 1:1 ratio. The tooth was then sealed with a temporary restoration. The procedure was performed three times at 1-month intervals.

The control group received conventional therapy, which included endodontic preparation of the root canals followed by extra-apical application of Metapex.

RESULTS AND DISCUSSION

The microflora of the apical granuloma contents is highly diverse, with a predominance of coccal forms. In addition to leukocytes and bacteria, this biological substrate contains desquamated epithelial cells, the number of which increases in the presence of inflammation.

Prior to treatment, high levels of microbial contamination of the apical granuloma contents were detected: Gram-positive cocci accounted for 2.8×10^7 CFU/mL, and Gram-negative cocci for 1.9×10^7 CFU/mL (Table 1). These values demonstrated statistically significant differences compared with the control group at all levels of significance.

According to the results of the microbiological analysis performed prior to treatment, high titers of pathogenic microorganisms were identified in the apical granuloma contents, including *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Tannerella forsythensis* (*Bacteroides forsythus*), *Prevotella intermedia*, and *Treponema denticola*.

Table 1. Microflora indicators of exudate of the periapical region of teeth animals main and control groups before treatment, CFU/ml

Таблица 1. Показатели микрофлоры экссудата периапикальной области зубов животных основной и контрольной групп до лечения, КОЕ/мл

Microorganisms	Periapical periodontitis without fistula formation ($n = 83$)
Gram-positive cocci	$2.8 \times 10^7 – 7.6 \times 10^7$
Gram-negative cocci	$1.9 \times 10^7 – 8.3 \times 10^8$

Table 2. Microflora indices of exudate from the periapical region of teeth in patients with periodontitis at different stages of treatment during the experiment, CFU/ml

Таблица 2. Показатели микрофлоры экссудата периапикальной области зубов у животных с периодонтитом на разных стадиях лечения в период проведения эксперимента, КОЕ/мл

Stage of treatment	Gram-positive cocci		Gram-negative cocci	
	Main group (n = 43)	Control group (n = 40)	Main group (n = 43)	Control group (n = 40)
Prior to treatment	2.8×10^7–$7.6\times10^{7*}$		1.9×10^7–$8.3\times10^{8*}$	
	$p = 0.234$		$p = 0.324$	
After treatment	$0.7\times10^{4**,***}$ $p < 0.001$	$1.2\times10^{5**}$ $p < 0.001$	$0.6\times10^{4**,***}$ $p < 0.001$	$1.1\times10^{5***}$ $p < 0.001$
	$p < 0.001$		$p < 0.001$	
6 months after completion of treatment	$0.9\times10^{4**,***}$ $p < 0.001$	$1.3\times10^{5**}$ $p < 0.001$	$0.8\times10^{5**,***}$ $p < 0.001$	$1.2\times10^{5*}$ $p = 0.018$
	$p < 0.001$		$p < 0.001$	
12 months after completion of treatment	$1.1\times10^{5**,***}$ $p < 0.001$	$1.6\times10^{5**}$ $p < 0.001$	$1.1\times10^{5*,***}$ $p = 0.048$	$1.7\times10^{5**}$ $p < 0.001$
	$p < 0.001$		$p < 0.001$	

Note. *, ** significance of differences compared with baseline values at $p < 0.05$ and $p < 0.001$, respectively (Wilcoxon test); *** indicates statistically significant differences between the main and the control groups (Mann–Whitney U test) at $p < 0.001$

Примечание. *, ** значимость различий показателей по сравнению с исходным значением при $p < 0{,}05$ и $p < 0{,}001$ соответственно (по критерию Вилкоксона); *** значимость различий показателей между основной и контрольной группами (согласно критерию Мана-Уитни) при $p < 0{,}001$

The findings demonstrated that, in animals with periapical periodontitis without fistula formation, microbial titers in the apical granuloma contents were elevated by 1–2 orders of magnitude ($p < 0.001$), indicating a substantial microbial load and its key role in the initiation and maintenance of the chronic inflammatory process in this condition.

Immediately following the proposed treatment protocol involving the stromal vascular fraction of adipose tissue and an osteogenesis stimulator, a statistically significant reduction in microbial counts was observed Gram-positive cocci and Gram-negative cocci decreased by 0.7×10^6 CFU/mL ($p < 0.001$). At 6 and 12 months after therapy, the results remained stable, with further reductions noted (Gram-positive cocci decreased by 0.5×10^6 CFU/mL and 0.3×10^6 CFU/mL, respectively ($p < 0.001$); Gram-negative cocci by 0.7×10^6 CFU/mL and 0.2×10^6 CFU/mL, respectively ($p < 0.05$)). These findings indicate a sustained, long-term therapeutic effect (Table 2).

After conventional baseline therapy, the control group demonstrated a reduction in microbial contamination titers of the apical granuloma contents by 0.2×10^5 ($p < 0.001$). However, after 6 months, microbial contamination returned to baseline levels. At 12 months post-treatment, a further increase in contamination was observed, by 0.3×10^6 CFU/mL ($p < 0.001$) (Table 2).

Intergroup differences in the microbiological parameters of apical granuloma contents in animals with apical periodontitis were statistically significant ($p < 0.001$) immediately after treatment and remained significant at both 6 and 12 months of follow-up, confirming the effectiveness of the proposed therapeutic approach.

In all groups, both the main and control cohorts, the level of microbial contamination demonstrated relatively high titers ranging from 2.9 to 3.7×10^6 CFU/mL. Course application of the stromal vascular fraction contributed to a statistically significant reduction in both Gram-positive and Gram-negative cocci by two orders of magnitude and one order of magnitude, respectively ($p < 0.001$), both immediately after treatment and during follow-up at 6 and 12 months. However, immediately after treatment, the number of Gram-positive cocci in Groups 1 and 2 was significantly lower ($p < 0.05$) compared with the control group. For Gram-negative cocci, a statistically significant reduction immediately after treatment in the main group compared with the control group ($p < 0.05$) was observed only in animals with apical periodontitis without fistula.

Thus, the high efficacy of the proposed endodontic treatment method for apical periodontitis using stromal vascular fraction of adipose tissue and an osteogenesis stimulator has been objectively demonstrated in the experimental model.

CONCLUSION

Analysis of long-term outcomes of treatment of apical destructive forms of periodontitis using the stromal vascular fraction and an osteogenesis stimulator in an experimental setting demonstrated a therapeutic efficacy of 99.9% of cases. The application of the stromal vascular fraction in combination with an osteogenesis stimulator in the management of apical periodontitis showed high effectiveness, contributing to a reduction in microbial contamination titers within the apical granuloma contents in experimental animals.

Evaluation of both immediate and long-term outcomes of the proposed treatment protocol based on the stromal vascular fraction and osteogenesis stimulator in an experimental model of apical periodontitis confirmed its high efficacy, a reduced incidence of complications, and a stable therapeutic effect in the long-term follow-up period.

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