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Original Article

Effect of minocycline combined with metronidazole on periodontitis and gingival crevicular fluid cytokines

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Abstract: Objective: To investigate the effect of minocycline combined with metronidazole on periodontitis and gingival crevicular fluid cytokines. Methods: A total of 92 patients with periodontitis treated in The Hospital of Zhejiang University Zijingang Campus from January 2017 to January 2018, were selected as research subjects. The patients were randomly divided into two groups: 43 patients (108 teeth) in the control group treated with minocycline, and 49 patients (108 teeth) in the observation group treated with minocycline and metronidazole. The probing depth (PD), plaque index (PLI), and gingival index (GI), percentages of spirochetes, cocci, and bacilli in subgingival adhering plaques, tumor necrosis factor-α (TNF-α), high-sensitivity C-reactive protein (hs-CRP), prostaglandin 2 (PGE2), interleukin 6 (IL-6) in the gingival crevicular fluid, and adverse reactions were compared between the two groups before and after treatment. Results: After treatment, PD, PLI, and GI were significantly improved compared with those before treatment in both groups, which were significantly less in the observation group than those in the control group (all $P<0.05$). The percentage of spirochetes, cocci, and bacilli in subgingival adhering plaques were significantly decreased after treatment in both groups than those before treatment, and those in the observation group were significantly lower than those in the control group (all $P<0.05$). After treatment, the levels of TNF-α, hs-CRP, PGE2, IL-6 in the gingival crevicular fluid were significantly lower than those before treatment in both groups, and those in the observation group were significantly lower than those in the control group (all $P<0.05$). The total clinical effective rate was 81.63% in the observation group, which was significantly higher than that in the control group (62.79%, $P<0.05$). No obvious adverse reactions occurred in either groups. Conclusion: The combination of minocycline and metronidazole in the treatment of periodontitis can synergistically enhance the antibacterial effect, reduce the level of cytokines in gingival crevicular fluid, and contribute to the reconstruction and recovery of periodontal tissues. This treatment is safe and worthy of clinical promotion.

Keywords: Periodontitis, minocycline, metronidazole, cytokine

Introduction

Periodontitis is an inflammatory disease caused by bacterial infection. It causes inflammatory destruction of the periodontal supporting tissue and becomes one of the leading causes of teeth loss in adults [1, 2]. For treatment of periodontitis, it is generally advocated to adopt antibacterial treatment as inhibiting growth of specific bacteria is the key to the treatment of periodontitis. Studies on the pathological mechanism of periodontitis show that inflammatory destruction caused by plaque microorganisms and their metabolic toxins is considered to be the root cause of the development and progression of periodontitis. The gingival crevicular fluid cytokines (tumor necrosis factor alpha (TNF-α), high-sensitivity C-reactive protein (hs-CRP), prostaglandin 2 (PGE2), interleukin 6 (IL-6), etc.) can mediate expansion and persistence of inflammatory responses and play an important immunomodulatory role in the destruction mechanisms of periodontium [3-5]. Enhancing the efficacy of antimicrobial treatment of periodontitis could significantly reduce the number and types of periodontal pathogens, decrease the level of cytokines in the gingival crevicular fluid, and improve the treatment efficacy.

Minocycline is widely used for the treatment of periodontitis and can achieve local sustained

release. It has advantages such as wide antibacterial spectrum, less drug-resistant bacteria, and promotion of periodontal tissue regeneration [6, 7]. A study has reported that there are many suspected pathogenic bacteria in periodontitis, in which Porphyromons gingivalis, Prevotella intermedia and Capnocytophaga are commonly observed. It is difficult to completely eradicate these pathogens by taking minocycline alone, which leads to a relatively high recurrence rate in the later stage [8]. Metronidazole is a commonly used antibiotic drug, with activity against anaerobic species that can effectively interfere with the growth and reproduction of bacteria, which is widely used to treat various types of periodontitis [9]. At present, it is still unclear whether minocycline combined with metronidazole can enhance anti-bacterial activity in the treatment of periodontitis, shorten the course of treatment and improve the curative effect for periodontitis, and relative research report is few. This study was conducted to observe the effect of local application of minocycline hydrochloride ointment combined with metronidazole on periodontitis and cytokines such as TNF-α, so as to provide experimental evidence for improving the clinical treatment of periodontitis.

Materials and methods

General information

A total of 92 patients with periodontitis, who were admitted to The Hospital of Zhejiang University Zijingang Campus from January 2017 to January 2018, were selected as the study subjects, in which the total number of teeth was 216. Among them, 55 cases were males and 37 cases were females, with an average age of 48.14 ± 3.92 years old. In this study, the patients were randomly divided into two groups: the observation group and the control group. In the observation group, 49 patients with 108 teeth were treated with minocycline and metronidazole. In the control group, 43 patients with 108 teeth were treated with minocycline alone. This study was approved by the Ethics Committee of The Hospital of Zhejiang University Zijingang Campus and all patients signed informed consent.

Inclusion criteria: all patients were in line with the diagnostic criteria of periodontitis and were older than 18 years old [10]; the total teeth number was larger than 20; probing depth (PD) was greater than 4 mm and combined with probing hemorrhage; gingival index (GI) was greater than 2; no antibacterial drugs and other anti-inflammatory drugs were used for treatment within 1 month; no local periodontitis treatment was taken within 6 months.

Exclusion criteria: allergic to minocycline and metronidazole; pregnancy or breastfeeding women; patient with acute pulpitis and periodontitis; combined with other systemic diseases such as cardiovascular and cerebrovascular diseases, diabetes, hepatic and renal dysfunction, etc.

Research methods

All patients were treated with basic periodontal treatment, including oral health education, guidance of the frequency, methods and times of tooth brushing, reasonable use of dental floss, and the supracondylar scaling, subgingival scaling, and root surface smoothing treatment were adopted.

For antibacterial treatment, minocycline treatment was adopted in the control group with details as follows. A special needle was used to gently insert minocycline hydrochloride ointment (Nippon Era Co., Ltd.) into the bottom of the pocket along the periodontal pocket wall. The ointment was lightly pushed into the pocket until the drug filled the periodontal pocket and overflowed to the gingival margin. The patients were enjoined not to eat, gargle, or brush within half an hour after applying the drug. The drug was administrated once a week for 4 weeks as a treatment course. The patients in the observation group were treated with metronidazole on the basis of the treatment in the control group. In order to administer the metronidazole, sterile forceps were used to hold a metronidazole stick (Sichuan Xicheng Pharmaceutical Co., Ltd.) into the periodontal pocket. The treatment was performed twice a week for 4 weeks as a treatment course. The efficacy was evaluated after 4 weeks of treatment in both groups.

Observation indicators

Comparison of PD, plaque index (PLI), and GI before and after treatment in the two groups: A periodontal examination was performed using the Florida periodontal probe, and PD, PLI and GI were measured according to the American

Table 1. Comparison of general data between two groups

	Case	Male/female	Age (year)	Course of disease (week)
Control group	43	26/17	48.64 ± 4.23	13.24 ± 2.81
Observation group	49	29/20	47.63 ± 3.71	14.43 ± 3.52
t/X^2		0.016	0.311	0.458
P		0.900	0.771	0.671

Table 2. Comparison of probing depth between two groups

	Case	Before treatment (mm)	After treatment (mm)	t	P
Control group	43	6.18 ± 0.41	4.49 ± 0.52	6.099	0.002
Observation group	49	6.17 ± 0.39	3.51 ± 0.19	4.042	0.010
t		0.031	4.351		
P		0.977	0.001		

Table 3. Comparison of plaque index between two groups

	Case	Before treatment	After treatment	t	P
Control group	43	2.23 ± 0.72	1.38 ± 0.36	2.538	0.035
Observation group	49	2.31 ± 0.67	0.97 ± 0.29	3.598	0.016
t		0.141	2.363		
P		0.895	0.036		

Society of Periodontology. In the measurement of PD, that was, the distance from the gingival margin to the bottom of the pocket or the bottom of the gingival sulcus, 6 sites were measured for each tooth and the mean value was adopted. In the PLI measurement, 4 dental faces were measured for each tooth and the average was taken. Using the periodontal probe, the dental faces were lightly scratched and scored according to the amount and thickness of the plaque: if there was no plaque in the gingival marginal area, the result was scored as 0; if there were thin plaques in the gingival marginal area and periodontal probe was needed to scrape off the plaque, scored as 1; if a moderate amount of plaque was seen on the gingival margin or adjacent surface, scored as 2; if there was a large amount of soft dirt in the gingival margin or adjacent surface, scored as 3. In the GI measurement, a periodontal probe was used in combination with the visual examination. Each tooth was measured on four tooth surfaces and the mean value was taken. The affected gingivae included in this study were examined: healthy gingiva, 0 score; mild gingival inflammation without bleeding during probing, 1 score; moderate gingival inflammation with bleeding during probing, 2 scores; severe gingival inflammation with automatic bleeding tendency, 3 scores.

Comparison of the percentage of spirochetes, cocci, and bacilli in subgingival adhering plaques between two groups of patients: A disinfected Florida periodontal probe was used to scrape the subgingival adhering plaques and perform negative staining. The staining agent was 2% Congo red aqueous solution. One hundred bacteria were counted and divided into cocci, bacilli, and spirochetes to calculate percentage of spirochetes, cocci, and bacilli in the subgingival adhering plaques.

Comparison of TNF-α, hs-CRP, PGE2 and IL-6 in gingival crevicular fluid before and after treatment in the two groups: The gingival crevicular fluid was collected using a filter paper strip and the filter paper was soaked in 100 μL of the eluent and then centrifuged to collect the eluent. After the above operation was repeated for 5 times, the TNF-α, hs-CRP, PGE2, and IL-6 in the eluent were measured by enzyme-linked immunosorbent assay strictly in accordance with the reagent operating instructions. Reagents were purchased from the US Science R&D company.

Comparison of the treatment efficacy of patients in the two groups: Using the chief complaints, clinical symptoms, and periodontal clinical indicators of the patients, the treatment efficacy was comprehensively evaluated. The judgment criteria for efficacy were as follows: excellent: patient's subjective symptoms disappears or significantly reduced, PD decreased by more than 2 mm or less than 3 mm, connective tissue attachment loss improved or unchanged, and bleeding index decreased by more than 1; effective: patient's subjective symptoms reduced, PD decreased by more than 1 mm but less than 2 mm, bleeding index decreased by more than 1 or PD decreases by more than 3 mm but the bleeding index is not

Table 4. Comparison of gingival index between two groups

	Case	Before treatment	After treatment	t	P
Control group	43	1.82 ± 0.55	1.36 ± 0.52	13.279	0.006
Observation group	49	1.92 ± 0.30	0.87 ± 0.20	33.126	0.001
t		0.276	3.036		
P		0.796	0.006		

Table 5. Comparison of spirochetes percentage between two groups (%)

	Case	Before treatment	After treatment	t	P
Control group	43	18.47 ± 0.69	12.05 ± 0.57	92.665	<0.001
Observation group	49	19.36 ± 0.58	6.53 ± 0.34	24.155	0.002
t		1.710	14.405		
P		0.162	<0.001		

Table 6. Comparison of cocci percentage between two groups (%)

	Case	Before treatment	After treatment	t	P
Control group	43	38.47 ± 0.69	24.95 ± 0.76	334.533	<0.001
Observation group	49	37.42 ± 1.02	12.02 ± 0.65	118.903	<0.001
t		1.477	22.394		
P		0.214	<0.001		

changed; ineffective: patient's subjective symptoms do not show any relief or even aggravate. The total clinical effective rate is calculated as follows: total clinical effective rate = (number of excellent + effective)/total case number * 100%.

Statistical methods

All data were processed using SPSS 19.0 software. The measurement data tallied with normal distribution was presented as mean ± standard deviation ($\bar{x}$ ± sd). The t-test was used for the comparison between groups. The paired t-test was used for the comparison of the indicators before and after treatment. The count data are expressed as percentages. The comparison between groups was performed using the X^2 test. The difference is statistically significant at P<0.05.

Results

Comparison of basic data between the two groups of patients

There were no statistically significant differences between the two groups in terms of gender, age, and course of disease, and they were comparable (all P>0.05). See **Table 1**.

Comparison of PD, PLI, and GI before and after treatment in the two groups

Before treatment, there was no significant difference in PD, PLI, and GI between the two groups (all P>0.05). After treatment, PD, PLI and GI in the two groups were significantly improved compared with those before treatment (all P<0.05); and the PD, PLI and GI were significantly lower in the observation group than those in the control group after treatment (all P<0.05). See **Tables 2**-**4**.

Comparison of microbiological indicators before and after treatment in the two groups

Before treatment, the percentage of spirochetes, cocci, and bacilli in the subgingival adhering plaques in both groups were not significantly different (all P>0.05). After treatment, the percentages of spirochete, cocci, and bacilli in the subgingival adhering plaques of both groups were significantly decreased than those before treatment (all P<0.05). The percentage of spirochetes, cocci, and bacilli in the subgingival adhering plaques was significantly lower in the observation group than those in the control group after treatment (all P<0.05). See **Tables 5**-**7**.

Comparison of cytokine levels in gingival crevicular fluid before and after treatment in both groups

Before treatment, the levels of TNF-α, hs-CRP, PGE2 and IL-6 in the gingival crevicular fluid of the two groups were not significantly different (all P>0.05). After treatment, the levels of TNF-α, hs-CRP, PGE2 and IL-6 in the gingival crevicular fluid were significantly lower than those before treatment in the two groups (all P<0.05). The levels of TNF-α, hs-CRP, PGE2 and

Table 7. Comparison of bacilli percentage between two groups (%)

	Case	Before treatment	After treatment	t	P
Control group	43	45.63 ± 1.25	33.95 ± 1.18	289.005	<0.001
Observation group	49	46.29 ± 1.04	21.83 ± 1.49	16.745	0.004
t		0.703	11.045		
P		0.521	<0.001		

Table 8. Comparison of tumor necrosis factor-α between two groups (μg/mL)

	Case	Before treatment	After treatment	t	P
Control group	43	2.68 ± 0.75	1.24 ± 0.40	8.314	0.014
Observation group	49	2.71 ± 0.64	0.69 ± 0.23	6.147	0.002
t		0.157	2.898		
P		0.883	0.016		

Table 9. Comparison of high-sensitivity C-reactive protein between two groups (μmol/mL)

	Case	Before treatment	After treatment	t	P
Control group	43	23.56 ± 1.48	13.57 ± 1.63	5.564	0.031
Observation group	49	24.16 ± 1.32	8.96 ± 0.54	33.753	<0.001
t		0.524	4.650		
P		0.628	0.010		

Table 10. Comparison of prostaglandin 2 between two groups (μg/mL)

	Case	Before treatment	After treatment	t	P
Control group	43	209.45 ± 27.54	168.98 ± 20.95	7.955	0.015
Observation group	49	211.36 ± 20.47	136.47 ± 16.60	68.270	<0.001
t		0.096	2.976		
P		0.928	0.014		

IL-6 in the gingival crevicular fluid after treatment were significantly lower in the observation group than those in the control group (all P<0.05). See **Tables 8**-**11**.

Comparison of clinical total effective rates in the two groups

The total clinical effective rate in the observation group was 81.63%, which was greater than the 62.79% in the control group. The difference was significant in the clinical total efficiency between the two groups (P = 0.043). See **Table 12**.

Analysis of adverse reactions in the two groups of patients

No obvious adverse reactions were observed in either group. Only one patient in the observation group developed gastrointestinal reactions, which was remitted without any special treatment, and did not affect follow-up treatment.

Discussion

Patients with periodontitis have different degrees of bad breath and gingival bleeding, which seriously threaten the physical and mental health of patients. Bacterial infections are the root cause of periodontitis. Because the morphology of the periodontal pocket and the anatomy of the teeth are relatively complex, the current use of mechanical treatment alone cannot effectively clean out the bacteria within it. Therefore, the effective control and elimination of bacteria is fundamental to the complete treatment of periodontitis. After systemic use of antibiotics, periodontal pockets have lower drug concentrations, more adverse reactions, and are more likely to induce bacterial drug resistance. For antimicrobial treatment of periodontitis, emphasis is currently placed on local sustained-release medications to avoid adverse reactions caused by systemic administrations. In addition, local medications are convenient and the patient compliance is better. In this study, all patients were treated with local antibacterial therapy. None of patients were non-cooperative during the procedure and no significant adverse reactions occurred. This was also consistent with the study results from Duarte et al. [11].

Minocycline hydrochloride has the advantages of wide antimicrobial activity spectrum, strong lipid solubility, and high safety of local medications [12]. It can also effectively prevent bone resorption and promote periodontal tissue regeneration [13, 14]. The results of this

Table 11. Comparison of interleukin 6 between two groups (μg/mL)

	Case	Before treatment	After treatment	t	P
Control group	43	5.31 ± 0.83	3.16 ± 0.66	4.744	0.001
Observation group	49	5.36 ± 0.75	1.86 ± 0.52	56.383	<0.001
t		0.077	3.787		
P		0.942	0.004		

Table 12. Comparison of total effective rate between two groups (case)

	Case	Excellent	Effective	Ineffective	Total effective rate (%)
Control group	43	13	14	16	62.79
Observation group	49	22	18	9	81.63
X^2					4.109
P					0.043

study showed that minocycline hydrochloride ointment treatment could significantly improve the periodontal measurement indicators (PD, PLI, GI, etc.) and reduce the proportions of bacterial strains such as spirochetes, cocci, bacillus, etc. in the subgingival adhering plaques, and inhibit cytokines produced by inflammatory cell in comparison of those before treatment. It can be seen that local application of minocycline hydrochloride in the treatment of periodontitis is effective. However, how to maximize the effect of periodontitis treatment through combination therapy has always been a difficult problem for dentists.

To increase the intensity of antibacterial treatment of periodontitis, reduce plaque formation, and microbial pathogenicity, and promote periodontal tissue reconstruction and repair, this study used metronidazole for combination therapy. Metronidazole mainly kills anaerobic microorganisms by inhibiting the formation of deoxyribonucleic acid in bacteria. One study has shown that superposition of the antagonistic effect of metronidazole for anaerobic bacteria, on the basis of antibacterial effect of minocycline hydrochloride ointment, makes significant progress in the antibacterial treatment of periodontitis [15]. The results of this study indicated that the total clinical effective rate in the observation group after combined treatment was 81.63%, while the total clinical effective rate in the control group with single use of minocycline hydrochloride was 62.79%. The difference between the two groups was statistically significant. Compared with the control group, the PD, PLI, GI and the percentage of spirochetes, cocci, and bacilli in the subgingival adhering plaques after treatment were significantly lower in the observation group than those in the control group, indicating that minocycline combined with metronidazole could synergistically enhance antibacterial effects in the treatment of periodontitis, and significantly improve clinical symptoms. Other studies have shown that the combination of minocycline and metronidazole can be used to treat periodontitis after basic periodontal treatment disrupted the bacterial biofilms. By alternative administration, the sensitivity of the bacteria to the drug was relatively high and the antibacterial effect was notable [16]. Guzeldemir et al. found that a large number of gram-negative anaerobic bacteria were present in the subgingival adhering plaques in patients with periodontitis and the triggered immune inflammatory response significantly increased the level of cytokines in the gingival crevicular fluid [17]. It can be seen that, for patients with periodontitis, the combination of metronidazole treatment on the basis of minocycline treatment can significantly enhance the antibacterial effect, and significantly reduce the inflammatory response.

Studies have shown that TNF-α, hs-CRP, PGE2, IL-6 in the gingival crevicular fluid can mediate the inflammatory response of periodontitis. They play regulatory roles in the immune inflammatory-based damage and can be used to evaluate the effects of antibiotics for periodontitis [18-21]. The results of this study show that the levels of TNF-α, hs-CRP, PGE2 and IL-6 in the gingival crevicular fluid after treatment are significantly lower in the observation group than those in the control group, and the difference was statistically significant; this might because of the insensitivity to minocycline hydrochloride ointment in part of the patients with periodontitis. Using the combination of minocycline hydrochloride ointment and metronidazole for treatment can effectively clean out the gram-negative

anaerobic bacteria in the subgingival adhering plaques and help to reduce the levels of cytokines in the gingival crevicular fluid. This further showed that the combination of the two drugs has a synergistic effect in the treatment of periodontitis.

In conclusion, the combination of minocycline and metronidazole in the treatment of periodontitis can synergistically enhance the antibacterial effect, reduce the level of cytokines in the gingival crevicular fluid, contribute to the reconstruction, recovery of periodontal tissues, and has good safety profile worthy of clinical generalization. However, this study has certain limitations such as small sample size, single-center research, etc. In the future studies, additional sample sizes and multi-center randomized clinical trials are needed for further confirmation.

Disclosure of conflict of interest

None.

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