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Original Article

Effect of tinidazole tablets combined with minocycline hydrochloride ointment on melatonin and IL-1β levels in patients with chronic periodontitis

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Abstract: Objective: This study aimed to investigate and compare the clinical effects of tinidazole tablets when given alone and combined with minocycline hydrochloride ointment (MHO) in patients with chronic periodontitis (CP), and the effects on melatonin and interleukin-1β (IL-1β). Methods: Seventy-six patients with CP in our hospital were included as the study subjects and divided into the study group (SG) (n = 38) and control group (CG) (n = 38) in accordance with a random number table. The patients in the SG were treated with tinidazole tablets combined with MHO. The patients in the CG were given tinidazole tablets only. The clinical efficacy and periodontal conditions were compared. The periodontal bleeding index (BI), gingival index (GI) and plaque index (PLI) were observed with periodontal probe. The incidence of adverse reactions and the changes of melatonin and IL-1β levels in gingival crevicular fluid were compared. Results: The effective cure rate in the SG was higher than that in the CG ($P = 0.032$). After treatment, BI, GI and PLI in the SG were lower than those in the CG ($P < 0.001$). They decreased after treatment ($P < 0.001$). There was no difference in the incidence of adverse reactions between the two groups ($P > 0.05$). The melatonin and IL-1β levels at T0 or T1 was not different between the two groups ($P > 0.05$). The melatonin and IL-1β levels at T2, T3 and T4 in the SG were lower than those in the CG ($P < 0.001$), which were the highest at T0 and the lowest at T4 in both groups ($P < 0.001$). Spearman correlation analysis showed that both the melatonin and IL-1β levels in the SG were negatively correlated with the treatment time ($P < 0.001$). Conclusions: Tinidazole tablets combined with MHO are more effective than tinidazole tablets alone in the treatment of CP. The melatonin and IL-1β levels can be remarkably reduced. This treatment combination is worthy of being popularized in the clinic in the future.

Keywords: Tinidazole tablets, minocycline hydrochloride ointment, chronic periodontitis, melatonin, IL-1β

Introduction

Chronic periodontitis (CP) is one of the most common oral diseases found clinically, and is also the most common progression of gingivitis [1]. Clinically, gingivitis can gradually develop into periodontitis. The disease usually invades the entire mouth and teeth [2]. CP can cause different degrees of systemic chronic inflammation. However, the inflammation usually occurs in the early stage of illness, which is often neglected by the patient due to its mild severity. Once there are clear clinical symptoms, the disease has become more serious [2, 3]. CP usually occurs in middle-aged and elderly population. However, recent studies have shown that younger populations with the disease have been trending [4]. CP can cause a series of periodontal diseases, such as teeth defects, gum destruction, necrosis, etc. As a result, the patient's normal life can be seriously influenced. What's more, oral cancer can be induced, which seriously threatens the patient's health and life [5, 6]. At present, tinidazole is mainly used in the treatment of CP. Tinidazole has strong antibacterial activity. It has significant advantages in the treatment of periodontitis caused by colony infection [7]. However, with the clinical application of tinidazole in recent years, its shortcomings are gradually showing up. If a large dose of the drug is needed, the possibility of drug resistance is increased; in

addition, the safety of it is low and the probability of adverse reactions is greatly increased [8]. Therefore, the research scholars are looking for new drugs to treat CP. Minocycline hydrochloride is a new drug discovered in recent years. As an anti-anaerobe agent, minocycline hydrochloride is not only highly safe but also has a significant therapeutic effect on CP [9]. This study speculated that tinidazole combined with minocycline hydrochloride ointment (MHO) can achieve significant results in the treatment of CP compared with tinidazole alone. The therapeutic significance was analyzed.

A study by Srinath, et al. [10] has found the presence of melatonin in patients with periodontitis. However, its exact role is not clear. As one of the key factors regulating immune and endocrine ability [11], the effect of interleukin-1β (IL-1β) on CP is also not clearly defined. Therefore, the conditions of melatonin and IL-1β during the treatment of CP were investigated in this study. Their relationship with CP was analyzed. Thus, a reliable reference is provided for the clinical diagnosis and treatment of periodontitis.

Materials and methods

General information

Seventy-six patients with CP in our hospital were selected as the study subjects, including 49 males and 27 females. The patients were aged 34-69 years, with an average age of (46.7±8.1) years. The patients were categorized into the study group (SG) (n = 38) and control group (CG) (n = 38) in accordance with a random number table. The patients in the SG received tinidazole tablets combined with MHO. The patients in the CG were given tinidazole tablets only. The study was approved by the Ethics Committee of Chengyang District People's Hospital. All subjects signed an informed consent form.

Inclusion/exclusion criteria

Inclusion criteria were as follows: patients who met the clinical manifestations of CP [12]; who were diagnosed with CP by a series of examinations in our hospital; who were aged 18-70 years; who had complete clinical data; who agreed to cooperate with the investigation and arrangement of medical and nursing staffs. Exclusion criteria are as follows: patients who were pregnant; who were complicated with other chronic diseases; who had tumors; who had autoimmune diseases; who had other cardiovascular, cerebrovascular or blood diseases; who had organ dysfunction, liver and kidney failure; who were allergic to drugs; who received other periodontal treatment within 3 months before admission; who had mental diseases; or who were transferred to another hospital.

Methods

After gum cleaning and curettage in both groups, the patients in the CG were treated with tinidazole tablets (Hainan Haili Pharmaceutical Group Co., Ltd., GYZZ H20046512) 1 g/time, once per day. The patients in the SG received MHO (Sunstar INC Japan, GYZZ H20100244) in addition to treatment given to the CG. The patient's oral cavity was covered by a rubber dam. After periodontal dryness, the ointment was injected once a week for 4 weeks. The gingival crevicular fluid was sampled before treatment (T0), at the 1st (T1), 2nd (T2), 3rd (T3) and 4th (T4) course of treatment. The levels of melatonin and IL-1β were determined with enzyme-linked immunosorbent assay (ELISA). The melatonin kit (QC9738-B) was purchased from Shanghai Qincheng Biotechnology Co., Ltd. IL-1β kit (JK-(a)-4956) was purchased from Shanghai Jingkang Bioengineering Co., Ltd. All tests were performed strictly in accordance with instructions of the kit in an aseptic environment.

Outcome measures

Clinical efficacy in both groups was measured. Significant improvement: the clinical symptoms disappeared. There was no pain or no feeling of tooth loosening. Improvement: after treatment, the clinical symptoms were effectively controlled. The loosening of teeth was alleviated. Patients can chew normally. Ineffective: after treatment, the clinical symptoms had no noticable changes. There was no remarkable pain. The scope of lesions was not reduced. The effective cure rate = (number of significant improvement + number of improvement)/total number ×100% was calculated.

Periodontal conditions in both groups: a periodontal probe was used to observe the periodontal bleeding index (BI), gingiva index (GI)

and plaque index (PLI) before and after treatment.

Adverse reactions in both groups: the adverse reactions during treatment were recorded. The incidence of adverse reactions was calculated = number of subjects with adverse reactions/ total number of subjects ×100%. Melatonin and IL-1β: the correlation between melatonin, IL-1β and treatment time was analyzed.

Statistical methods

All experimental results were statistically calculated with SPSS24.0 (Shanghai Yuchuang Network Technology Co., Ltd.). Graphpad8 (Softhead Inc.) was used for plotting of all figures and the secondary checking of calculation for results. The enumeration data, such as clinical efficacy, incidence of adverse reactions, etc., were expressed as number/rate [n (%)]. Chi-square test was used for comparison among groups. The measurement data, such as melatonin and IL-1β levels, were represented as mean ± standard deviation (SD). The t-test was adopted for comparison among groups. Repeated measurement variance analysis and Bonferroni method were introduced for comparison among multiple time points. Spearman coefficient of correlation was used for correlation analysis. $P < 0.05$ implied a significant difference.

Results

Comparison of general information

There was no difference in age, BMI, course of disease, full-mouth remnant of teeth, gender, smoking, drinking, edible areca-nut, marital status, living conditions, degree of education, history of tooth brushing, ethnicity and molar between the two groups ($P > 0.05$), which were comparable between the two groups (**Table 1**).

Comparison of clinical efficacy

The SG reported an effective rate of 91.67%, which was significantly higher than that of 72.22% in the CG ($P = 0.032$, **Table 2**).

Comparison of periodontal conditions

There was no difference in BI, GI or PLI between the two groups before treatment ($P > 0.05$). After treatment, BI, GI and PLI decreased in both groups ($P < 0.001$) as compared with the conditions before treatment, and the SG had significantly lower metrics than the CG (**Figures 1-3**).

Comparison of adverse reactions

The incidence of adverse reactions was 8.33% in the SG and 16.67% in the CG ($P > 0.05$), which had no significant difference (**Table 3**).

Comparison of melatonin and IL-1β

There was no difference in melatonin and IL-1β levels between the two groups ($P > 0.050$) at T0 or T1. The melatonin and IL-1β levels at T2, T3 and T4 in the SG were significantly lower than those in the CG ($P < 0.001$), which were the highest at T0 and the lowest at T4 in both of the groups (**Figures 4** and **5**).

Correlation between melatonin, IL-1β and treatment time

Spearman correlation analysis showed that both melatonin and IL-1β levels in the gingival crevicular fluid were negatively correlated with the treatment time ($P < 0.001$) (**Figures 6** and **7**; **Table 4**).

Discussion

Periodontitis mainly includes gingivitis, periodontal trauma, and periodontal atrophy, etc. [13]. During the course of CP, the absorption capacity of the alveolar bone is increased. Meanwhile, periodontal damage is serious, resulting in tooth defects [14]. At present, the pathogenesis of CP has reached a consensus at home and abroad. It may be related to dental plaque, traumatic occlusion and hematological disease [15, 16]. In addition, many other risk factors also contribute to the increased morbidity of CP [17]. As there is no significant breakthrough in the early diagnosis of CP at present, most patients have developed into the middle and late stage by the time of diagnosis [18]. As a result, the treatment is more difficult. Therefore, the research scholars constantly strive to find new treatments [19]. As a highly strong antibacterial drug, minocycline hydrochloride has an excellent effect in the treatment of CP. In this study, relatively remarkable results were obtained in the treatment of CP with tinidazole tablets combined with MHO. This is of great sig-

Table 1. Comparison of general information [n (%)]

	Study group (n = 36)	Control group (n = 36)	t or X^2	*P*
Age	45.8±9.2	46.2±8.8	0.189	0.851
BMI (kg/m^2)	24.21±3.84	24.49±4.05	0.301	0.764
Course of disease (years)	2.07±0.41	2.12±0.52	0.453	0.652
Full-mouth remnant teeth	22.08±2.84	22.14±2.43	0.096	0.924
Gender			0.575	0.448
Male	26 (72.22)	23 (63.89)		
Female	10 (27.78)	13 (36.11)		
Smoking			0.064	0.801
Yes	25 (69.44)	24 (66.67)		
No	11 (30.56)	12 (33.33)		
Drinking			0.241	0.624
Yes	22 (61.11)	24 (66.67)		
No	14 (38.89)	12 (33.33)		
Edible areca-nut			0.072	0.789
Yes	27 (75.00)	26 (72.22)		
No	9 (25.00)	10 (27.78)		
Marital status			2.057	0.152
Married	34 (94.44)	36 (100.00)		
Unmarried	2 (5.56)	0 (0.00)		
Living conditions			0.956	0.326
Town	29 (80.56)	32 (88.89)		
Village	7 (19.44)	4 (11.11)		
Degree of education			0.241	0.624
< High school	12 (33.33)	14 (38.89)		
≥ High school	24 (66.67)	22 (61.11)		
History of tooth brushing			0.465	0.496
Yes	30 (83.33)	32 (88.89)		
None	6 (16.67)	4 (11.11)		
Ethnicity			1.059	0.304
Han	33 (91.67)	35 (97.22)		
Minority	3 (8.33)	1 (2.78)		
Molar			0.605	0.437
Yes	12 (33.33)	9 (25.00)		
No	24 (66.67)	27 (75.00)		

Table 2. Comparison of clinical efficacy [n (%)]

	Study group (n = 36)	Control group (n = 36)	X^2	*P*
Significant improvement	20 (55.56)	12 (33.33)		
Improvement	13 (36.11)	14 (38.89)		
Ineffectiveness	3 (8.33)	10 (27.78)		
Effective cure rate (%)	91.67	72.22	4.614	0.032

nificance for clinical diagnosis and treatment of CP in the future.

Our study results showed that the effective cure rate in the SG was dramatically higher than that in the CG, which indicated that tinidazozle tablets combined with MHO were more effective than tinidazole tablets alone in the treatment of CP. The cure rate was basically consistent with the results of a study by Jhinger, et al. [20]. Minocycline hydrochloride mainly contains dimethylamine tetracycline, which has a strong inhibitory effect on anaerobes in the oral cavity [21]. This is the key for the treatment of CP. Although tinidazole also has an anti-anaerobe effect and plays a stable role in the elimination of local inflammation

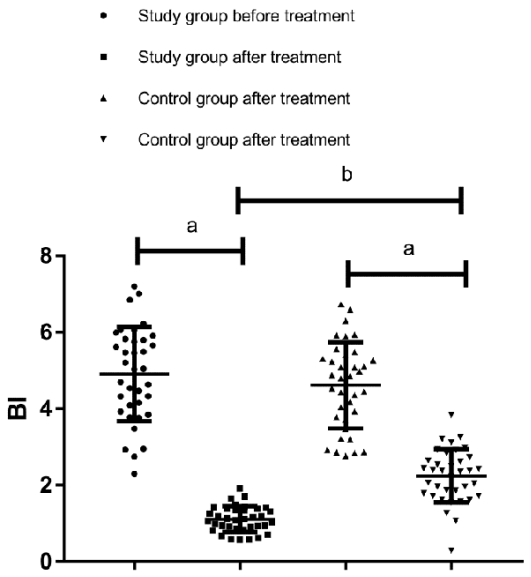

Figure 1. Comparison of BI before and after treatment in the two groups. 'a' indicated $P < 0.001$ compared with the BI before treatment in the same group. 'b' implied $P < 0.001$ compared with the BI after treatment in the SG.

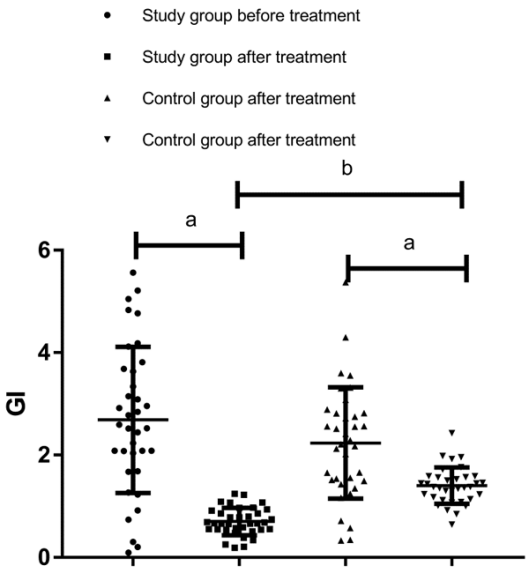

Figure 2. Comparison of GI before and after treatment. 'a' indicated $P < 0.001$ compared with the GI before treatment in the same group. 'b' implied $P < 0.001$ compared with the GI after treatment in the SG.

caused by anaerobes, the patient's resistance to the drug may be highly influential due to the limitation of its antibacterial spectrum [22] and its long-term use as the first choice for treatment of CP. Therefore, the sterilization effect in

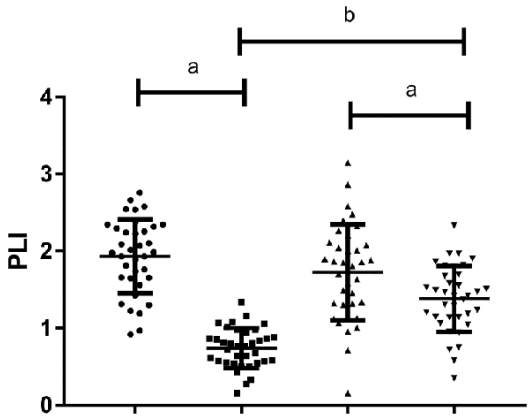

Figure 3. Comparison of PLI before and after treatment. 'a' presented $P < 0.001$ compared with the PLI before treatment in the same group. 'b' suggested $P < 0.001$ compared with the PLI after treatment in the SG.

the CG was not as remarkable as that in the SG. Minocycline hydrochloride not only has a very wide antibacterial spectrum, but clinically also has a very few known drug-resistance bacteria. During the killing of germs, anaerobes can be synthesized to produce proteins [23]. Minocycline hydrochloride not only has significant therapeutic effect, but also plays a certain role in improving the health of periodontal and dental tissues. Minocycline hydrochloric has inhibitory effects on enzyme activity [24]. It can reduce the attachment among periodontal tissues and decrease the alveolar resorption. Thus, the possibility of adverse reactions in the course of treatment is also greatly reduced. In this study, the incidence of adverse reactions in the SG was not statistically different from that in the CG. However, the actual number of subjects was much lower than that in the CG. The result may be due to the statistical calculation error caused by the small number of patients. The sample size will be enlarged as soon as possible to validate this viewpoint. The comparison of BI, GI and PLI between the two groups showed that these periodontal measures in the SG were remarkably lower than those in the CG after treatment. It also further proved the significant effect of minocycline hydrochloride plus tinidazole in the treatment of CP. This study speculated that it may be related to the usage of MHO. MHO can be kept for a long time in the periodontal tissues by filling. In this process, the drug can not only play a therapeutic effect, but also keep the periodon-

Table 3. Comparison of adverse reactions [n (%)]

	Study group (n = 36)	Control group (n = 36)	X^2	*P*
Dizziness	0 (0.00)	1 (2.78)		
Nausea and vomiting	1 (2.78)	1 (2.78)		
Skin allergy	1 (2.78)	0 (0.00)		
Gastrointestinal discomfort	0 (0.00)	2 (5.56)		
Fever	1 (2.78)	1 (2.78)		
Dizziness	0 (0.00)	1 (2.78)		
Incidence of adverse reactions (%)	8.33	16.67	1.143	0.285

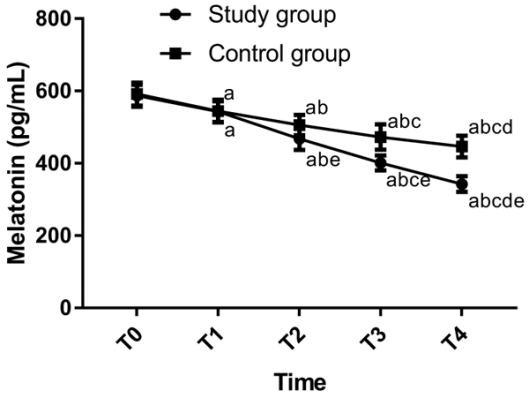

Figure 4. Comparison of melatonin during treatment between the two groups. 'a, b, c' and 'd' respectively implied $P < 0.001$ compared with the melatonin at T0, T1, T2 and T3 in the same group. 'e' indicated $P < 0.001$ compared with the melatonin in the same period in the SG.

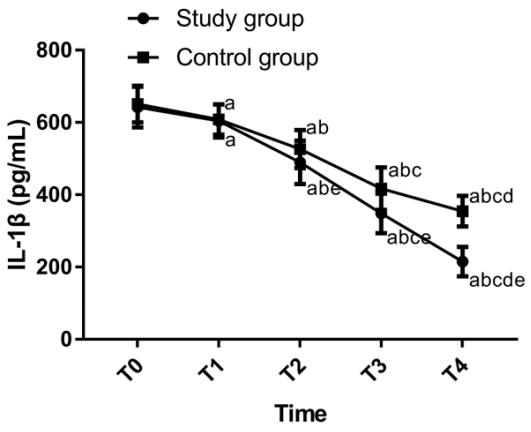

Figure 5. Comparison of IL-1β during treatment between the two groups. 'a, b, c' and 'd' respectively represented $P < 0.001$ compared with the IL-1β at T0, T1, T2 and T3 in the same group. 'e' suggested $P < 0.001$ compared with the IL-1β in the same period in the SG.

tal tissue healthy and stable. In this state, the periodontal ligament cells gradually transform

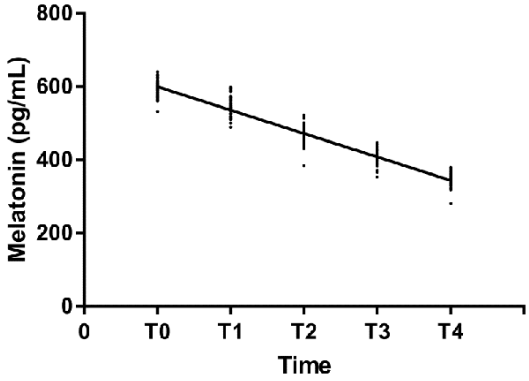

Figure 6. Correlation between melatonin and treatment time in the SG. Spearman correlation analysis showed that the melatonin level in gingival crevicular fluid in the SG was negatively correlated with the treatment time ($r = -0.961$, $P < 0.001$).

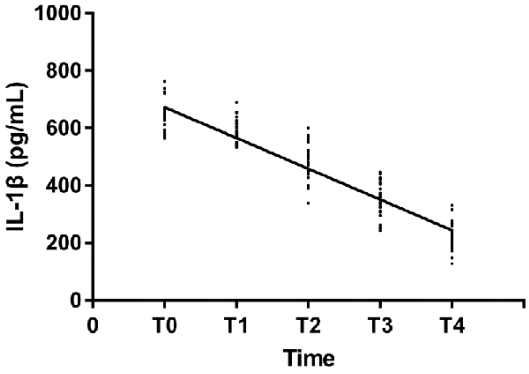

Figure 7. Correlation between IL-1β and treatment time. Spearman correlation analysis showed that the IL-1β level in the SG had a negative correlation with the treatment time ($r = -0.937$, $P < 0.001$).

into the bone cells. Thus, the recovery process can be accelerated.

Further comparison of melatonin and IL-1β between the two groups showed that the melatonin and IL-1β levels in gingival crevicular fluid in the SG were significantly lower than those in

Table 4. Correlation between melatonin and IL-1β levels in gingival crevicular fluid and treatment time in the SG

	Melatonin	IL-1β
r	-0.961	-0.937
95% CI	-0.971~-0.948	-0.953~-0.915
P	< 0.001	< 0.001

the CG at T2, T3 and T4. Melatonin and IL-1β in the SG were negatively correlated with the treatment time. It implied that they were closely related to the progression of CP. As a very active substance, melatonin not only has antioxidant effects, but also promotes the formation of bone tissues by bone cells [25]. In this study, melatonin decreased dramatically after treatment, which indicated that the more serious the periodontitis was, the higher the level of melatonin was. The reason may be that the periodontal tissue was damaged after periodontitis. At this time, the metabolites formed by melatonin under the attack of bacteria further promoted the occurrence of inflammation. This is also consistent with the results of Mehrzadi, et al. [26] in human bone marrow mesenchyme stem cells. IL-1β is involved in the inflammatory response and cartilage destruction and absorption by stimulating T and B lymphocytes. Some studies have shown that IL-1β can inhibit the expression of alkaline phosphatase in periodontal ligament fibroblasts. It is not conductive to the healing of periodontal tissues [27]. Therefore, the remarkable decrease of IL-1β in the SG in this study also implied the clinical application value of tinidazole tablets combined with minocycline hydrochloride.

This study aimed to investigate the effect of tinidazole tablets combined with MHO in the treatment of CP. However, there are still some deficiencies due to the limited test conditions. As no basic animal experiments are performed, the exact mechanism of tinidazole tablets combined with MHO in treatment of CP cannot be determined. The prognosis of patients cannot be estimated due to the short period of the study. The application of tinidazole tablets combined with minocycline hydrochloride should be further analyzed. Meanwhile, the sample size and investigation period will be expanded to obtain the optimal study results.

In summary, tinidazole tablets combined with MHO is more effect than tinidazole tablets alone in the treatment of CP and can significantly reduce the levels of melatonin and IL-1β, which is worthy of being popularized clinically.

Disclosure of conflict of interest

None.

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