

Effect and treatment of lactobacillus on inflammation around the implant

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Abstract: Ultrasonic scaling and antibiotic therapy are traditional therapeutic methods for inflammation around the implant but therapeutic effect is not ideal. In view of maintaining flora balance around the implant and implant long-term solid holdup, this experiment observes impact and clinical effect of lactobacillus metabolite on inflammation around the impact to explore a new kind of ecological drug. This drug has little or no side effect, good curative effect and low recurrence rate, which can be applied for broad groups of people. 16 cases with inflammation around the impact were divided into experimental group and control group, 8 cases for each group. Lactobacillus metabolites gargle was offered to experimental group; purified water was offered to control group. Gargle way is 3 times/day, 20ml/time, 3min/time and for 7 days. Two groups of cases were clinical and microbiological tested before gargle, 3 days, 7 days and 30 days after gargle. Based on clinical and microbiological test of 8 cases of health implant, we observe sub gingival flora variation trend and clinical effects of infectors with inflammation around implant. Lactobacillus metabolite can improve clinical index of inflammation around the impact including MPLI, GI, MBI and PD. Lactobacillus metabolite has a strong treatment effect on inflammation around the implant and has no side effect.

Keywords: Lactobacillus metabolite; inflammation around the impact; effect; treatment

INTRODUCTION

In recent 30 years, artificial dental implant technology has been developing rapidly in developed countries. Artificial implant with bone fusion what is also called denture have become a kind of effective retention and support equipment of dental restoration and a regular restoration method of denture loss and defect (Quirynen *et al.*, 2002). Application prospect of oral implant is very positive but meanwhile failure of minority implant exists. Inflammation around the implant is collectively called implant and pathological state of tissue around it. It is a kind of infectious disease that induces by bacteria. Pathogenic bacteria destroy soft tissue closed barrier and synostosis interface around implant by bacteria surface material, toxin and metabolite. Then clinical symptoms such as soft tissue inflammation around the implant increase of probing depth, bleeding, abscess pain on probing, loose of implant and bone resorption would appear and lead to implant failure. Prevention and treatment method of inflammation around the implant is to restore physiological combination of flora around the implant by adjust balance of flora around implant. Lactobacillus is oral normal flora. It was proved that lactic acid around lactobacillus metabolites can reduce PH value of surroundings and control growing of acidophilic bacteria in gingival sulcus. This experiment observes composition, amount and clinical index change of sub gingival flora before and after using lactobacillus metabolite gargle on patients with inflammation around the implant to evaluate curative effect of lactobacillus

metabolite on inflammation around the implant. It aims to explore a new kind of ecological drug, which have little or no side effect, good curative effect and low recurrence rate and can be applied for broad groups of people.

Main body

In recent years, biological materials and artificial organ are more and more widely applied in Medical area. Internal implant is a branch, which develops fastest and has the largest influence. It has become one of four major breakthrough of oral scientific development in 20 century with high-speed turbine, panoramic radiograph X-ray machine and macromolecule concentration material. Medical experts at home and abroad find that gram-negative bacillus is conditioned pathogen of inflammation around the implant by analysis of patients with inflammation around the implant. Therefore, control of sub gingival gram-negative bacillus of implant is critical to treatment of inflammation. Traditional treatment of inflammation around the implant is consisting of ultrasonic scaling an antibiotic therapy. However, combining with patients' condition, these two kinds of treatment are not ideal. On contrary, ultrasonic scaling will cause obvious scratch in the meanwhile of cleaning bacterial plaque and tartar. It will damage oxidation protective layer on surface of implant and then affect biocompatibility and corrosion resistance of implant, which is beneficial for secondary accumulation of bacterial plaque on rough surface of implant. Effect of antimicrobial treatment is not ideal. Human body is generally drug resistance to antibacterial agent and effect of pharmacy on patients with inflammation around is not good. Long terms of application of antibacterial drug lead

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to inhibition of beneficial bacterium in oral cavity and gastrointestinal tract. Pathogenic bacterium and opportunistic pathogen produce drug resistance and excessive multiply, which lead to the imbalance of flora and damage of internal environment ecological balance in human body (Buchmann *et al.*, 2003).

Modern oral microbiology theory holds that inflammation around the implant is a kind of flora imbalance disease. Low immunity, chemical stimulus, mechanical injury and large dose of antibiotics will lead to position transfer and host transfer. Change of ingredients and ratio of sub gingival flora transform sub gingival flora from physiological combination into pathological combination (Broggini *et al.*, 2003). According to this theory, approach of prevention and treatment of inflammation around the implant is to restore physiological combination of flora of implant by adjust balance of flora around the implant

Diagnostic criteria of inflammation around the implant

Clinical diagnosis criteria: So far, there is divergence in formulation of diagnosis criteria of inflammation around the implant.

Criteria of Mombelli are the most popular. Its main content is to do clinical examine and X ray text on patients at regular intervals two weeks after implant operation. Clinical criterion is: pocket depth ≥ 4 mm and gingival index ≥ 1 . Diagnosis criterion of X ray is: height of alveolar crest lowers for no less than 3mm.

MATERIAL AND METHODS

Other equipment and reagent

Liquid paraffin, collarium, L wave bar, disposable oral cavity appliance box, alcohol lamp, human serum, palladium particles.

Research object

Referring to Mombelli's diagnosis criteria on inflammation around the implant, we chose 8 cases with health implant and 16 cases with inflammation around the implant. Out of 24 cases, half were female and the other half were male; they aged from 21~55 years. There are totally 24 implants. Those who used cylindrical implant, have finished fixed denture for more than 6 month and have no dentition defect and missing, diabetes and other systemic diseases, have not taken antibiotic and immunosuppressor in three month and have no treatment of periodontal and periodontal cultivation in three month are selected (Allan *et al.*, 2002). The study has been approved by medical ethics committee and all patients have signed informed consent.

Therapeutic process

16 cases with inflammation around the implant are randomly brought into experimental group and control

group, 8 for each group. Lactobacillus metabolites gargle was offered to experimental group; purified water was offered to control group. Gargle is performed 3times/ day, 20ml/time, 3min/time and for 7 days. Package of gargle is the same. Two groups of cases were respectively clinical and microbiological tested before gargle, 3 days, 7 days and 30 days after gargle. Taking it as a standard, we observe clinical effect and changing trend of sub gingival flora of patients who are with inflammation around the implant.

Primary instrument and appliance

Name of appliance	Production corporation
Electric heating constant temperature	Jinbei Vacuum Instruments Factory of Tianjin HH- B11
Incubator	Yuejin Medical Apparatus and Instruments Factory of Shanghai GZX-DH X
Electrothermal blowing	Purifying Equipment Factory of Suzhou
Sterile purification platform	Medical Apparatus and Instruments Factory of Dalian
Anaerobic jar	OLYMPUS
Microscope	Haier Corporation
General refrigerator	French Gilson
Finnpipette	
Periodontal probe vernier caliper Sterile	
Paper point	

Main reagent and culture medium

Lactobacillus metabolite	Lab of some medical university
BHI-S	BECTON DICKINSON
Chlorhematin	Hunan Yiyang Yiwei Co., Ltd
Defiber protein sheep protein	Animal room of some medical university
Neomycin sulfate	AMRESCO
Vancomycin	Eli Lilly Japan K.K
Trypan Blue	Chemical reagent of sinopharm
Bacitracin	SIGUMA

Clinical test

Clinical Index

MPLI: 0 means no bacterial plaque; 1 means bacterial plaque can be found only when probe tip sweep over the surface of implant and bacteria plaque value in surface of rough implant that is sprayed by thick liquid is at least 1. 2 means invisible bacterial plaque; 3 means large amount of material Alba.

GI: 0 refer to normal gingiva; 1 means gingiva have little edema and probe tip can not make it bleeding; 2 means gingiva have little edema and probe tip can make it bleeding; 3 means gingiva have a trend of spontaneous bleeding or anabrosis.

MBI: 0 means no bleeding when probing along gingival margin; 1 means scattered punctate hemorrhage; 2 means linear distribution of bleeding in gingival sulcus; 3 means severe bleeding.

PD: distance from bottom of periodontal pocket to gingival margin. Adopt 0.2 N of power when measure.

Diagnostic criteria of inflammation around the implant: so far, it is controversial in formulation of diagnosis criteria of inflammation around the implant, among which criteria of Mombelli is the most popular. Its main content is to make a clinical test 2 weeks after patients put on implant denture. Its clinical criteria are periodontal $PD \geq 4\text{mm}$ and $GI \geq 1$ (Tangli et al., 2002).

Evaluation of curative effect: its criteria can be classified into two grades according to criteria of Mombelli. Recovery: $PD \leq 2\text{mm}$, $GI \leq 1$; Invalid: $PD \geq 5\text{mm}$, $GI \geq 2$, and even with pyorrhea of pocket and fistula.

Microbiology examination

Confirmation of sub gingival bacterial plaque amount

Serum of normal people that is similar to GCF is taken as specimen. Take 0.1, 0.2, 0.3, 0.4, 0.5, 1.7, 1.8, 1.9, 2.0ul of serum by finnpipette whose range is 2ul on sterile paper point. Measure its wetted length by vemier caliper. Measure three sterile paper points on each point and draw standard curve with its average value. Afterwards, get GCF by same sterile paper point. Find out the relative GCF on that standard curve according to the wetted length.

Collection of specimen

Collect specimen from 8:30am to 10:00am. Before collection, subjects should gargle by warm water. Supragingival bacterial plaque should be stroke off. Wet lap. Insert sterile paper point into gingival sulcus in mesial buccal site of dental implant by sterile forceps and take it out 10 seconds later. Measure the wetted length of sterile paper point by vemier caliper. Put it into centrifuge tube, which is contained with 0.5ml of mercaptoethanol acid salt delivery liquid and lid with liquid paraffin to inspect as soon as possible (Xiaowei et al., 2002).

Attenuation of specimen

Shock and disperse the specimen fully and dilute it by 10 times series. Take 0.2ml of specimen stoste and add it into 1.8ml of Acid cysteine diluent and intensively mix up. Take 0.2ml of mixed liquor (10-1) into another 1.8ml of acid cysteine and dilute in turn according to the method. Dilution degree of general gingival sulcus bacteria plaque is 10-1-10-2 and aseptic technique is requested in dilution process.

Inoculation and culture of specimen

Take 50ul stoste, 10-1 and 10-2 each and inoculate in fresh prepared BHI-S, FS agar, MS agar, MSB agar and

LBS agar. Smear evenly by sterile glass rod. Put it into anaerobic jar and add reducing agent palladium particles. Place it for anaerobic culture (10%CO₂, 10%H₂, and 80% N₂) under the temperature of 37°C for 5-7 days.

Identification and count of sub gingival bacteria plaque

Select black or brownish black single colony whose diameter is 1 mm in BHI-S and observe them under microscope after staining (Xiaomin, 2008). Colony with negative bacillus may be objective colony. Make a biochemical identification after enriching fungus. Bacteria which show up negative Gram staining results may be the targeted colony; they are biomedically identified after enrichment. Bacteria which show negative result in sugar fermentation experiment are Porphyromonas gingivalis and bacteria showing positive in both sugar fermentation experiment and indole experiment are Prevotella intermedia. The two bacteria are counted. Moreover, Fusobacterium nucleatum, oral streptococci strain, Streptococcus mutans and Lactobacillus are counted respectively after being confirmed under microscope.

STATISTIC ANALYSIS

SPSS 10.0 software is used to make rank sum test on clinical index of every implant and sub gingival flora in and between groups. Difference is considered to be statistically significant if $P < 0.05$.

RESULT

Treatment of DM 9811 metabolite on inflammation around the implant comparison of clinical index before and after treatment of experiment group

MPLI, GI, MBI and PD of implant in experiment group are all downward 3, 7, 30 days after treatment, and there is a statistically significant difference ($P < 0.05$) compared with before treatment. Difference is statistically significant 7 days and 3 days after treatment ($P < 0.05$), but is not significant 7 days and 30 days after treatment ($P > 0.05$). Details are shown in table 1:

Comparison of clinical index after treatment of control group

MPLI, GI, MBI and PD of control experiment are not significantly different ($P > 0.05$) before treatment and 3 days, 7 days, 30 days after treatment, as showed in table 2:

Inspection result of clinical index of health implant comparison of clinical index change before and after treatment

MPLI of experimental and control group are not significantly different ($P > 0.05$) before treatment. But 3, 7 and 30 days after treatment, the difference is statistically significant ($P < 0.05$) and moreover experimental group is lower than control group. MPLI in experimental group is not significantly different with health group 7 and 30 days after treatment.

Table 1: Comparison of clinical index before and after treatment in experimental group

Clinical index	Before pharmacy			3 days after pharmacy			7 days after pharmacy			30 days after Pharmacy		
	P25	M	P75	P25	M	P75	P25	M	P75	P25	M	P75
MPLI	2.00	2.50	3.00	1.00	1.50	2.00	0.25	1.00	1.750	0	1.00	1.750
GI	2.00	2.00	2.00	1.00	1.00	1.00	0	0.250	0.750	0	0.375	1.00
MBI	1.250	1.875	2.00	1.00	0.875	1.00	0	0.375	1.00	0	0.375	1.00
PD (mm)	4.098	4.245	4.398	2.299	2.663	3.023	1.658	1.846	2.040	1.658	1.841	2.00

Table 2: Comparison of clinical index before and after treatment in control group

Clinical index	Before pharmacy			3 days pharmacy			After 7 days Pharmacy			After 30 days Pharmacy			After
	P25	M	P75	P25	M	P75	P25	M	P75	P25	M	P75	P75
MPLI	2.00	2.50	3.00	2.00	2.50	3.00	2.00	2.50	3.00	2.00	2.50	3.00	3.00
GI	1.250	1.875	2.00	1.250	1.875	2.00	1.00	1.625	2.00	1.250	1.875	2.00	2.00
MBI	1.250	2.00	2.750	1.00	1.875	2.750	1.00	1.750	2.00	1.00	1.875	2.750	2.750
PD (mm)	4.055	4.168	4.238	4.075	4.169	4.238	4.083	4.174	4.238	4.078	4.178	4.250	4.250

GI in experimental and control group is not significantly different before treatment, but is significantly different 3, 7 and 30 days after treatment ($P < 0.05$). Moreover, experimental group is lower than control group. GI of experimental group is not significantly different with health group 7 and 30 days after treatment.

Table 3: List of clinical index examination of health implant

	MPLI	GI	MBI	PD (mm)
P25	1	0	0	1.263
M	1.125	0.375	0.250	1.569
P75	1.750	1	0.750	1.913

MBI of experimental and control group are not significantly different before treatment ($P > 0.05$), but is significantly different 3, 7 and 30 days after treatment ($P < 0.05$) and experimental group is lower than control group. MBI of experimental group is not significantly different with health group 7 and 30 days after treatment.

PD of experimental and control group is not significantly different before treatment ($P > 0.05$), but is significantly different 3, 7 and 30 days after treatment ($P < 0.05$). And experimental group is lower than control group. PD of experimental group is not significantly different with health group 7 and 30 days after treatment.

Side reaction

There is no side reaction in experimental and control group.

DISSUSSION

Lactobacillus DM 9811 metabolite can improve clinical index of inflammation around implant. We find that lactobacillus DM 9811 metabolite have an effective

therapeutic effect on inflammation around the implant through observation of clinical index. Clinical index such as MPLI, GI, MSB and PD are all improved when the preparation is used. Clinical symptom such as increase of PD, bleeding of probing, abscess all disappears. Its curative effect is significant and not easy to relapse. Improved effect of clinical index in experimental group is basically corresponding to that of health implant 7 days after treatment. 30 days after treatment, clinical index do not have obvious change, side reaction and relapse tendency. It may be related to adherency and distribution of bacterial plaque around the implant. Lactobacillus DM 9811 metabolite gargle is a kind of ecological preparations, which will not lead to injure and drug resistance of implant. On the one hand, acid environment is beneficial for dissolution of calcium in bacterial plaque, reducing amount of bacteria around implant and improving clinical index; on the other hand, it can inhibit gram-negative anaerobic bacteria around implant, damage formation of plaque biofilm and interrupt adherency of bacteria plaque, which can adjust balance of subgingival flora radically around the implant and maintain clinical curative effect of inflammation around the implant (Yingliang *et al.*, 2009).

To sum up, lactobacillus metabolite gargle have an obvious effect on inflammation around the implant. It may become a new method of curing inflammation around the implant or health care product of preventing inflammation around the implant which is used in regular mouthwash before and after implant operation (Jianlei *et al.*, 2010). As an ecological preparation, action mechanism of lactobacillus metabolite is to adjust imbalance of flora that is caused by various reasons. It does not have toxic and side effect and possess advantage in clinical application, which have a great development prospect.

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