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Original Article

Novel regimens of phytopolyphenols with cisplatin or memantine and $ZnSO_4$ for synergistic inhibition of growth and gingipains of the cultured *Porphyromonas gingivalis*

Yu-Feng Huang [a,b†], Hui-Wen Yang [a,b†], Shoei-Yn Lin-Shiau [a,c*]

[a] *School of Dentistry, College of Oral Medicine, Chung Shan Medical University, Taichung, Taiwan*
[b] *Department of Stomatology, Chung Shan Medical University Hospital, Taichung, Taiwan*
[c] *Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan*

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Abstract *Background/purpose: Porphyromonas gingivalis* (P.g.) played a keystone pathogen not only in initiation and progression of periodontitis but also as a risk factor involved in systemic diseases (Alzheimer's disease, cancers, diabetes, osteoporosis etc.). Developments of effective and safe drugs to inhibit P.g. growth are urgent. In this study, we aimed at approaching novel regimens so called (PTM) by combination of repurposing drugs including phytopolyphenols (P) (curcumin, tea polyphenols), targeting drugs (T) such as cisplatin or memantine and metal ions(M) ($ZnSO_4$).
Materials and methods: The synergistic (combination Index (CI) < 1) antiproliferation and anti-protease efficacies (IC50) of novel regimens on cultured P.g. were evaluated by OD600 and colorimetric method respectively.
Results: The results obtained revealed that these novel regimens (PTM) synergistically (combination index, CI < 1) exerted not only antiproliferative but also anti-gingipain protease effects of P.g. The concentrations for 50% inhibition (IC50) of novel regimens on P.g. growth and gingipains were greatly decreased as compared with those of cisplatin and memantine alone.
Conclusion: Since these novel regimens exerted potent anti-bacterial effects on both planktonic and biofilm P.g., it is encouraged for further preclinical and clinical trials.

* Corresponding author. School of Dentistry, College of Oral Medicine, Chung Shan Medical University, Taichung, 40201, Taiwan.
E-mail address: syshiau@csmu.edu.tw (S.-Y. Lin-Shiau).
† These authors equally contributed to this paper.

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Introduction

Porphyromonas gingivalis (P.g.) is known not only as a keystone pathogen of periodontal diseases[1,2] but also involved in the etiology of systemic diseases[3–5] such as cancers, atherosclerosis, osteoporosis etc., especially Alzheimer's disease (AD).[6–8] Thus, investigation of effective and safe novel regimens against P.g. growth especially against P.g. biofilm is urgent. In our previous experiments, tea polyphenols were found to synergistically enhance the inhibitory effect of memantine against neuroexcitotoxicity.[9,10] Furthermore, both tea polyphenols and curcumin possessed antibacterial effects. Thus, in this study, we attempted to explore the novel regimens not only against P.g. growth but also anti-protease activities of P.g. The strategy to design compositions of novel regimens was to combine phytopolyphenols (P) and targeting drugs (T), either cisplatin[11–13] (cis, the commonly used anticancer drug) or memantine[14–16] (mem, for therapy of moderate AD) and metal ions (M) such as $ZnSO_4$[17–19] (antibacterial and immune-modulating agent), so called PTM regimens. We investigated these PTM regimens on the growth and gingipain protease inhibition of planktonic and biofilm P.g. The results obtained indicated the synergistic effects of these novel regimens against P.g. growth and protease activities. These novel regimens appeared to be promising and encouraging for further preclinical and clinical investigations.

Materials and methods

Drugs studied

Curcumin(C) was purchased from Merck Co. (Darmstadt, German). Green tea polyphenols purified from *Camellia sinensis* containing 98% polyphenols (75% catechins HPLC and 50% EGCG HPLC) similar to polyphenol E[20] was purchased from Hunan Huacheng Biotech, Inc. China. Cisplatin and memantine were from Sigma Chemical Company (St. Louis, Missouri, USA).

Culture of *Porphyromonas gingivalis*

The anaerobic pathogen, *Porphyromonas gingivalis*, (P.g.), ATCC 33277 was cultured in LB (Luria–Bertani) broth supplemented with hemin (5 μg/ml) and menadione (1 μg/ml) at 37 ± 0.5 °C in the anaerobic chamber containing 10% H_2, 5% CO_2 and 85% N_2, Forma Scientific Inc., Marietta, OH, USA).[21]

Anti-microbial effects of drugs on the proliferation of *Porphyromonas gingivalis (P.g.)*

The proliferation rate of the cultured P.g. was measured by Elisa reader (BioTek's Epoch™ Micro-Volume Spectrophotometer System) at optical density 600 nm (OD600).[21] After 16 h culture, the OD600 value of cultured P.g. was adjusted to about 0.1. The effects of the drugs on P.g. proliferation was evaluated by the changes of OD600 after addition of various concentrations of drugs (10 μl/well) to 90 μl/well of the cultured bacteria diluted with cultured broth by fold at OD600 about 0.06 in 96 well microplate. The drug effects were evaluated after 24 h incubation with the bacteria by the changes of OD600 and then calculated as percentage of the control treated with vehicle saline. The experiments were carried out in triplicate. The antibacterial potency of the drugs was quantitatively by the concentration inhibition curves and calculated the concentration of each drug for 50% inhibition (IC50).[21]

The synergistic antimicrobial effect of various drug combinations as compared with that of drug alone were assessed by combination index (CI).[22]

$$CI = \frac{(IC_{50})_1\ in\ combination}{(IC_{50})_1\ alone} + \frac{(IC_{50})_2\ in\ combination}{(IC_{50})_2\ alone} + \frac{(IC_{50})_3\ in\ combination}{(IC_{50})_3\ alone}$$

$$CI < 1, synergism; CI = 1, addition; CI > 1, antagonism$$

$$Potency\ ratio(fold) = \left(\frac{(IC_{50})_1\ alone}{(IC_{50})_1\ in\ combination} + \frac{(IC_{50})_2\ alone}{(IC_{50})_2\ in\ combination} + \frac{(IC_{50})_3\ alone}{(IC_{50})_3\ in\ combination}\right) \times \frac{1}{3}$$

Table 1 Antiproliferative effects (IC50) of novel regimens of cisplatin and memantine on the cultured planktonic *Porphyromonas gingivalis* (P.g.).

Drugs	IC50 (μg/ml·μg/ml·μM)	CI	Potency
C	113.1 ± 8.6		
GC	87.3 ± 9.1·87.3 ± 9.1		
Cis	10.6 ± 1.0		
Mem	146.6 ± 8.5		
Zn	443.3 ± 16.7		
CCis	65 ± 2.8·2.2 ± 0.1	0.8	3.3
GCCis	57.4 ± 2.4·57.4 ± 2.4·1.8 ± 0.1	0.8	3.6
CMem	59.2 ± 1.4·59.2 ± 1.4	0.9	2.2
GCMem	50 ± 2.3·50 ± 2.3·50 ± 2.3	0.9	2.3
CisZn	>5·50	>0.6	<5.5
MemZn	128.8 ± 10.6·43.1 ± 3.6	1.0	5.7
CZn	104 ± 10.2·34.6 ± 3.4	1.0	6.9
GCZn	67.5 ± 4.0·67.5 ± 4.0·20.6 ± 1	0.8	11.4
CCisZn	49.5 ± 2.3·1.4 ± 0.2·15.2 ± 1	0.6	12.9
GCCisZn	48 ± 1.8·48 ± 1.8·1.6 ± 0.2· 16.1 ± 0.6	0.7	12.1
CMemZn	49.5 ± 2.1·49.5 ± 2.1· 15.4 ± 0.7	0.8	11.4
GCMemZn	42.8 ± 2.3·42.8 ± 2.3· 42.8 ± 2.3·14.2 ± 0.7	0.8	12.2

C, curcumin; GC, green tea polyphenols plus curcumin; Cis, cisplatin; Mem, memantine; Zn, $ZnSO_4$.

Table 2 Antibiofilm formation (IC50) of novel regimens of cisplatin and memantine on the cultured *Porphyromonas gingivalis* (P.g.).

Drugs	IC50 (μg/ml•μg/ml•μM)	CI	Potency
C	122.9 ± 7.9		
GC	92.7 ± 7.0•92.7 ± 7.0		
Cis	44.9 ± 3.4		
Mem	175.5 ± 11.2		
Zn	438.2 ± 15.6		
CCis	77 ± 3.7•2.6 ± 0.2	0.7	9.6
GCCis	90.4 ± 8.9•90.4 ± 8.9• 2.8 ± 0.2	1.0	8.4
CMem	78.2 ± 1.9•78.2 ± 1.9	1.1	1.9
GCMem	62.8 ± 3.1•62.8 ± 3.1• 62.8 ± 3.1	1.0	2.1
CisZn	>5•50	>0.2	<8.9
MemZn	245.7 ± 47.9•81.9 ± 16	1.6	3.0
CZn	111.5 ± 5.3•37.2 ± 1.8	1.0	6.4
GCZn	85.7 ± 7.2•85.7 ± 7.2• 26.3 ± 2.1	1.0	8.9
CCisZn	104.9 ± 1.2•3.2 ± 0.1• 32.4 ± 0.8	1.0	9.7
GCCisZn	94.4 ± 3.4•94.4 ± 3.4• 3.2 ± 0.1·31.5 ± 1.1	1.2	9.7
CMemZn	59.6 ± 4.9•59.6 ± 4.9• 18.6 ± 1.4	0.9	9.5
GCMemZn	67.6 ± 5.7•67.6 ± 5.7• 67.6 ± 5.7·22.4 ± 1.8	1.2	7.9

C, curcumin; GC, green tea polyphenols plus curcumin; Cis, cisplatin; Mem, memantine; Zn, $ZnSO_4$.

Effects of drugs on biofilm formation of *Porphyromonas gingivalis (P.g.)*

The cultured P.g. was incubated with various concentrations of drugs anaerobically at 37 ± 0.5 °C for 48 h. The effects of drugs on biofilm formation were evaluated by changes of OD600 values.[23,24] Concentrations of drugs for 50% inhibition (IC50) and their synergistic effects were assessed as described previously.[22]

Effects of drugs on arg-gingipain protease activities of *Porphyromonas gingivalis (P.g.)*

After drug treatments for 48 h on P.g. biofilm formation, the protease activities of P.g. biofilm was assayed by the hydrolysis of the protease substrate, 0.5 mM N-α-benzoyl-arg-p-nitroanilide at 37 °C for 30 min. The protease activities were evaluated by measurement of colored product at OD405 as described.[24,25]

Statistics

Results for each experiment were represented as mean ± SEM. One way ANOVA followed by a post-hoc t test was used to evaluate differences between the groups. The level of significance was defined as $P < 0.05$.

Results

Tables 1 and 2 described the potencies (IC50) of drugs either alone or in combinations on the inhibitions of the proliferations of planktonic or biofilm formation of cultured *Porphyromonas gingivalis* (P.g.) respectively. Curcumin(C), tea polyphenols plus curcumin (GC), cisplatin (Cis), memantine (Mem) and zinc ($ZnSO_4$) all inhibited P.g. proliferation in both planktonic and biofilm nearly equally except that Cis was less potent about 4 times on biofilm formation as compared to that on planktonic P.g. (Tables 1 and 2). The important findings were that the combination of phytopolyphenols (C or GC) with either Cis or Mem exhibited synergistic antiproliferative effects (CI < 1) on both planktonic and biofilm P.g. respectively. Furthermore, two of those novel regimens plus $ZnSO_4$ (C•Cis•Zn and GC•Cis•Zn) enhanced synergistically (CI < 1) much better antiproliferative potencies to about twelve and nine times on planktonic and biofilm P.g. respectively (Tables 1 and 2).On the other hand, ratios of IC50 values of Cis used alone over those in the novel regimens were mostly within four and fourteen times on the planktonic and biofilm P.g. respectively (Fig. 1A), suggesting that the antiproliferative potencies of Cis were greatly increased and thus the

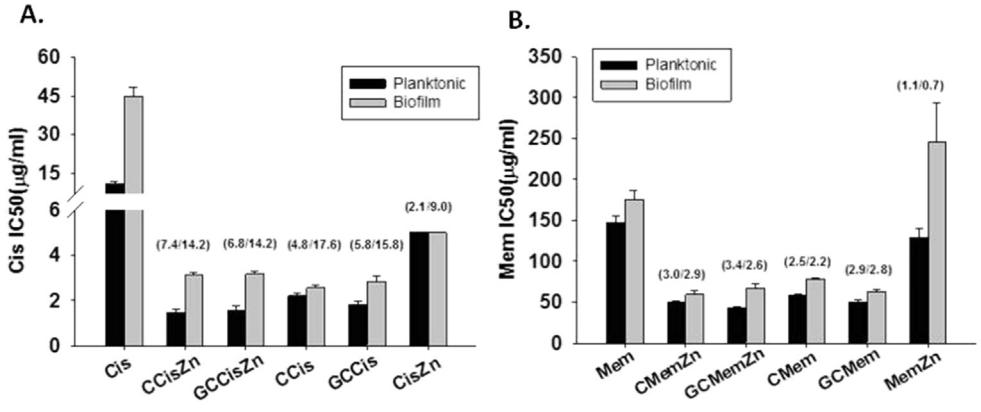

Figure 1 Ratio of IC50 of cisplatin (A) and memantine (B) used alone over those in the novel regimens for anti-proliferative effects and antibiofilm formation of cultured *Porphyromonas gingivalis* (P.g.).

Table 3 Anti-protease effects (IC50) of novel regimens of cisplatin and memantine on the cultured *Porphyromonas gingivalis* (P.g.) biofilm.

Drugs	IC50 (μg/ml•μg/ml•μM)	CI	Potency
C	245 ± 19.1		
GC	63.5 ± 0.5·63.5 ± 0.5		
Cis	418.0		
Mem	340.7 ± 4.6		
Zn	437.6 ± 2.0		
CCis	66.7·2.2	0.3	96.8
GCCis	48.9 ± 0.1·48.9 ± 0.1·1.6 ± 0.0	0.8	131.3
CMem	71.9 ± 6.3·71.9 ± 6.3	0.5	4.1
GCMem	37.5 ± 1.7·37.5 ± 1.7· 37.5 ± 1.7	0.7	5.4
CisZn	>5·50	>0.1	<46.2
MemZn	>150·50	>0.6	<5.5
CZn	81.4 ± 3.6·27.1 ± 1.2	0.4	9.6
GCZn	60.2·60.2·19.9	1.0	11.5
CCisZn	66.6 ± 5.2·2.1 ± 0.1·21.9 ± 1.7	0.3	75.9
GCCisZn	52.9·52.9·1.8·17.6	0.9	86.1
CMemZn	79.6 ± 1.8·79.6 ± 1.8· 26.3 ± 0.7	0.6	8.0
GCMemZn	53.3 ± 5.5·53.3 ± 5.5 · 53.3 ± 5.5·17.8 ± 1.9	1.0	10.7

C, curcumin; GC, green tea polyphenols plus curcumin; Cis, cisplatin; Mem, memantine; Zn, $ZnSO_4$.

concentrations of Cis could be greatly decreased which would certainly increase the safety and decreased the side effects of Cis.

Similarly, the novel regimens of Mem, also synergistically (CI < 1) or additionally (CI = 1) enhanced the anti-proliferative effects on planktonic and biofilm P.g. respectively (Tables 1 and 2). The ratio of IC50 of Mem used alone over those in novel regimens were within 2–3 times on planktonic and biofilm P.g. respectively (Fig. 1B).

Table 3 indicated IC50 of all drugs either alone or novel regimens in anti-protease effects on biofilm P.g. Most of the novel regimens also exhibited synergistic anti-protease effects (CI < 1). The ratios of IC50 of Cis (Fig. 2A)and Mem (Fig. 2B) used alone over those in novel regimens mostly were greater than 200 times and 4 times respectively, with the exception of Cis·Zn and Mem·Zn.

Discussion

In this study, we have successfully composited the novel regimens containing phytopolyphenols (curcumin,C; tea polyphenols,G) and cisplatin (C•Cis, GC•Cis) or memantine (C·Mem,GC•Mem) or in additional with $ZnSO_4$ (C•Cis (Mem)·Zn; GC•Cis (Mem)•Zn) for antibacterial agents. These regimens exhibited potent inhibitions on the growth of the keystone pathogen *Porphyromonas gingivalis* (P.g.), which could orchestrate the host-commensal microbiota homeostasis into dysbiosis, leading to the initiation and progression of the chronic inflammatory periodontal diseases.[1,2] Thus, the effective and safe drugs to combat P.g. growth are urgently needed. Fortunately, the novel regimens studied in this paper showed promising not only synergistic antibacterial effects but also anti-gingipain protease activities of P.g. Considering these repurposing drugs being effective and safe, it is encouraged to explore the possibility of these novel regimens for clinical managements of periodontitis, cancer and Alzheimer's diseases.

In our laboratory, we have studied the biological effects of phytopolyphenols (curcumin, tea polyphenols etc.) for more than 30 years.[26–29] Both curcumin and tea polyphenols possessed antioxidant, antiinflammatory, anticarcinogenicity, immune-modulatory, antibacterial and neuroprotective effects. Moreover, both curcumin[30–33] and tea polyphenols[34,35] were shown to be the promising antibacterial agents for the therapy of periodontitis. Thus, these natural products combined with Cis or Mem especially further combined with $ZnSO_4$[17–19] (an antibacterial and immunomodulatory agent) against P.g. growth were proofed to be promising novel regimens.

Most importantly, these phytopolyphenols inhibited gingipain protease activities of P.g., especially they synergistically inhibited these enzyme activities in the novel regimens. Since gingipains were important virulence factors of P.g. to subvert the host immune complement responses and for

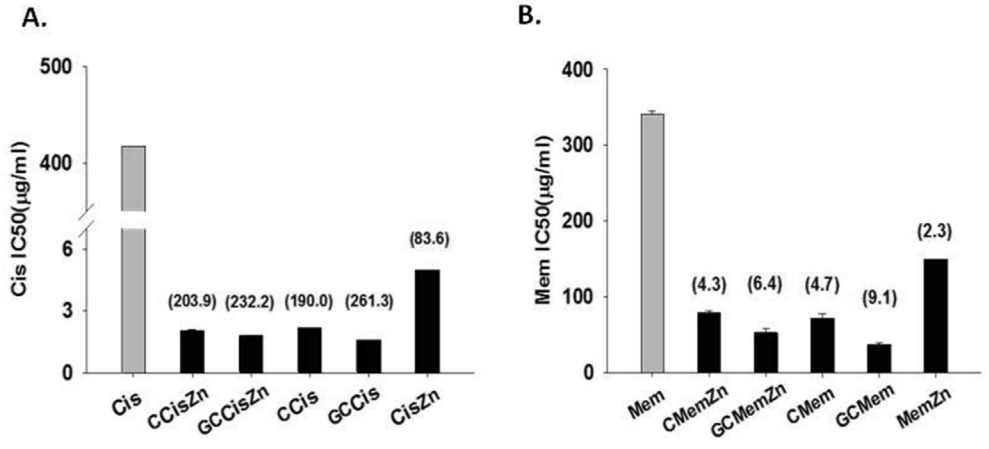

Figure 2 Ratio of IC50 of cisplatin (A) and memantine (B) used alone over those in the novel regimens for anti-protease activity of *Porphyromonas gingivalis* (P.g.) biofilm.

inducing inflammatory and proteolytic hydrolysis of connective tissues to provide nutritional supplements to themselves as well as to the opportunistic pathogens in the polymicrobial synergy dysbiosis.[36,37] These findings further confirmed their potentiality of novel regimens to clinical applications.

In conclusion, developments of antibacterial agents on P.g. are urgent for therapeutic managements of periodontitis and many systemic diseases (cancer, Alzheimer's disease etc.). The novel regimens studied in this paper apparently revealed the advantages as P.g. antagonists including: (1) All drugs are repurposing both effective and safe. (2) The synergistic effects of the drugs in the novel regimens in both antibacterial and anti-protease activities. (3) Multiple targeting regimens and pleiotropic pharmacological effects may combat the problem of occurring drug resistance. These preliminary results merits for further preclinical and clinical investigations.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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References

1. Fiorillo L, Cervino G, Laino L, et al. Porphyromonas gingivalis, periodontal and systemic implications: a systematic review. *Dent J* 2019;7:114.
2. Olsen I, Lambris JD, Hajishengallis G. Porphyromonas gingivalis disturbs host-commensal homeostasis by changing complement function. *J Oral Microbiol* 2017;9:1340085.
3. Binder Gallimidi A, Fischman S, Revach B, et al. Periodontal pathogens Porphyromonas gingivalis and Fusobacterium nucleatum promote tumor progression in an oral-specific chemical carcinogenesis model. *Oncotarget* 2015;6:22613—23.
4. Ha NH, Park DG, Woo BH, et al. Porphyromonas gingivalis increases the invasiveness of oral cancer cells by upregulating IL-8 and MMPs. *Cytokine* 2016;86:64—72.
5. Lin FY, Huang CY, Lu HY, et al. The GroEL protein of Porphyromonas gingivalis accelerates tumor growth by enhancing endothelial progenitor cell function and neovascularization. *Mol Oral Microbiol* 2015;30:198—216.
6. Costa MJF, de Araújo IDT, da Rocha Alves L, et al. Relationship of Porphyromonas gingivalis and Alzheimer's disease: a systematic review of pre-clinical studies. *Clin Oral Invest* 2021;25:797—806.
7. Elwishahy A, Antia K, Bhusari S, Ilechukwu NC, Horstick O, Winkler V. Porphyromonas gingivalis as a risk factor to Alzheimer's disease: a systematic review. *J Alzheimers Dis Rep* 2021;5:721—32.
8. Tang Z, Liang D, Cheng M, et al. Effects of Porphyromonas gingivalis and its underlying mechanisms on Alzheimer-like tau hyperphosphorylation in Sprague-Dawley rats. *J Mol Neurosci* 2021;71:89—100.
9. Chen CM, Lin JK, Liu SH, Lin-Shiau SY. Characterization of neurotoxic effects of NMDA and the novel neuroprotection by phytopolyphenols in mice. *Behav Neurosci* 2010;124:541—53.
10. Chen CM, Lin JK, Liu SH, Lin-Shiau SY. Novel regimen through combination of memantine and tea polyphenol for neuroprotection against brain excitotoxicity. *J Neurosci Res* 2008;86:2696—704.
11. Le Tourneau C, Tao Y, Gomez-Roca C, et al. Phase I trial of Debio 1143, an antagonist of inhibitor of apoptosis proteins, combined with cisplatin chemoradiotherapy in patients with locally advanced squamous cell carcinoma of the head and neck. *Clin Cancer Res* 2020;26:6429—36.
12. Li WZ, Lv X, Hu D, et al. Effect of induction chemotherapy with paclitaxel, cisplatin, and capecitabine vs cisplatin and fluorouracil on failure-free survival for patients with stage IVA to IVB nasopharyngeal carcinoma: a multicenter phase 3 randomized clinical trial. *JAMA Oncol* 2022;8:706—14.
13. Routila J, Qiao X, Weltner J, et al. Cisplatin overcomes radiotherapy resistance in OCT4-expressing head and neck squamous cell carcinoma. *Oral Oncol* 2022;127:105772.
14. Broberg DN, Wong D, Bellyou M, et al. Effects of memantine and high dose vitamin D on gait in male APP/PS1 Alzheimer's disease mice following vitamin D deprivation. *J Alzheimers Dis* 2022;85:1755—66.
15. Smolyarchuk EA, Leykin ZN. Comparative clinical study of the pharmacokinetics and bioequivalence of the combined drug Mioreol and the combined use of mono-drugs containing donepezil and memantine. *Zh Nevrol Psikhiatr Im S S Korsakova* 2022;122:85—91.
16. Souchet B, Audrain M, Alves S, et al. Evaluation of memantine in AAV-AD rat: a model of late-onset Alzheimer's disease predementia. *J Prev Alzheimers Dis* 2022;9:338—47.
17. Babayevska N, Ł Przysiecka, Iatsunskyi I, et al. ZnO size and shape effect on antibacterial activity and cytotoxicity profile. *Sci Rep* 2022;12:8148.
18. Dhawan M, Emran TB, Priyanaka, Choudhary OP. Immunomodulatory effects of zinc and its impact on COVID-19 severity. *Ann Med Surg (Lond)* 2022;77:103638.
19. Faghfouri AH, Khabbazi A, Baradaran B, et al. Immunomodulatory and clinical responses to zinc gluconate supplementation in patients with Behçet's disease: a double-blind, randomized placebo-controlled clinical trial. *Clin Nutr* 2022;41:1083—92.
20. Chow HH, Cai Y, Hakim IA, et al. Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. *Clin Cancer Res* 2003;9:3312—9.
21. Pourhajibagher M, Chiniforush N, Ghorbanzadeh R, Bahador A. Photo-activated disinfection based on indocyanine green against cell viability and biofilm formation of Porphyromonas gingivalis. *Photodiagnosis Photodyn Ther* 2017;17:61—4.
22. Chou TC. Drug combination studies and their synergy quantification using the Chou-Talalay method. *Cancer Res* 2010;70:440—6.
23. Wang HY, Lin L, Tan LS, Yu HY, Cheng JW, Pan YP. Molecular pathways underlying inhibitory effect of antimicrobial peptide Nal-P-113 on bacteria biofilms formation of Porphyromonas gingivalis W83 by DNA microarray. *BMC Microbiol* 2017;17:37.
24. Kariu T, Nakao R, Ikeda T, Nakashima K, Potempa J, Imamura T. Inhibition of gingipains and Porphyromonas gingivalis growth and biofilm formation by prenyl flavonoids. *J Periodontal Res* 2017;52:89—96.
25. Dashper SG, Pan Y, Veith PD, et al. Lactoferrin inhibits Porphyromonas gingivalis proteinases and has sustained biofilm inhibitory activity. *Antimicrob Agents Chemother* 2012;56:1548—56.
26. Lin JK, Pan MH, Lin-Shiau SY. Recent studies on the biofunctions and biotransformations of curcumin. *Biofactors* 2000;13:153—8.

27. Lin JK, Lin-Shiau SY. Mechanisms of cancer chemoprevention by curcumin. *Proc Natl Sci Counc Repub China B* 2001;25:59—66.
28. Lin JK. Cancer chemoprevention by tea polyphenols through modulating signal transduction pathways. *Arch Pharm Res (Seoul)* 2002;25:561—71.
29. Lin JK, Lin-Shiau SY. Mechanisms of hypolipidemic and anti-obesity effects of tea and tea polyphenols. *Mol Nutr Food Res* 2006;50:211—7.
30. Guimaraes-Stabili MR, de Aquino SG, de Almeida Curylofo F, et al. Systemic administration of curcumin or piperine enhances the periodontal repair: a preliminary study in rats. *Clin Oral Invest* 2019;23:3297—306.
31. Akpinar A, Calisir M, Cansın Karakan N, Lektemur Alpan A, Goze F, Poyraz O. Effects of curcumin on alveolar bone loss in experimental periodontitis in rats: a morphometric and histopathologic study. *Int J Vitam Nutr Res* 2017;87:262—70.
32. Xiao CJ, Yu XJ, Xie JL, Liu S, Li S. Protective effect and related mechanisms of curcumin in rat experimental periodontitis. *Head Face Med* 2018;14:12.
33. Asteriou E, Gkoutzourelas A, Mavropoulos A, Katsiari C, Sakkas LI, Bogdanos DP. Curcumin for the management of periodontitis and early ACPA-positive rheumatoid arthritis: killing two birds with one stone. *Nutrients* 2018;10:908.
34. Ramasamy C. Potential natural antioxidants: adjuvant effect of green tea polyphenols in periodontal infections. *Infect Disord: Drug Targets* 2015;15:141—52.
35. Varoni EM, Lodi G, Sardella A, Carrassi A, Iriti M. Plant polyphenols and oral health: old phytochemicals for new fields. *Curr Med Chem* 2012;19:1706—20.
36. Guevara T, Rodríguez-Banqueri A, Lasica AM. *Structural determinants of inhibition of Porphyromonas gingivalis gingipain K by KYT-36, a potent, selective, and bioavailable peptidase inhibitor*9; 2019:4935.
37. Eckert M, Mizgalska D, Sculean A, Potempa J, Stavropoulos A, Eick S. *In vivo expression of proteases and protease inhibitor, a serpin, by periodontal pathogens at teeth and implants*33; 2018:240—8.