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Resveratrol decreases local inflammatory markers and systemic endotoxin in patients with aggressive periodontitis

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Abstract

Background: Inflammation is hypothesized to contribute to the pathogenesis of periodontitis. Resveratrol (RV) is known for its anti-inflammatory properties. The purpose of this study was to investigate the inhibitory effect of RV on local inflammatory markers and systemic endotoxin in patients with periodontitis.

Methods: A total of 160 patients with periodontitis were enrolled in this study. The selected patients were randomly divided into four groups and received placebo, high-dose (500 mg/d) of RV (HRV, n = 40), middle-dose (250 mg/d) of RV (middle dose RV (MRV), n = 40) and low-dose (125 mg/d) of RV (low dose RV (LRV), n = 40) with orally administration. All patients received an 8-week treatment. The periodontal status of patients with periodontitis was recorded by using clinical attachment level (CAL), bleeding index (BI), oral hygiene index-simplified (OHI-S), and probing pocket depth (PPD). The levels of inflammatory markers in serum and gingival crevicular fluid (GCF), and systemic levels of endotoxin were evaluated using high sensitivity enzyme-linked immuno sorbent assay.

Results: Outcomes showed that symptoms of periodontitis determined by CAL, BI OHI-S and PPD were improved by RV compared to placebo. RV treatment decreased inflammatory markers in serum and GCF compared to placebo in patient with periodontitis. Systemic endotoxin declined more in the RV group than the placebo-treated group.

Conclusion: In conclusion, data in the current study indicate that RV is an efficient drug for the treatment of patients with periodontitis. The findings of the present study find that RV inhibits systemic local inflammatory markers and systemic endotoxin and suggest that 500 mg/d RV is the ideal dose for patients with periodontitis.

Abbreviations: BI = bleeding index, CAL = clinical attachment level, CRP = C-reactive protein, GCF = gingival crevicular fluid, GMCSF = granulocyte-macrophage colony stimulating factor, HRV = high dose RV, IL-1β = interleukin-1alpha, IL-10 = interleukin-10, IL-12p40 = interleukin-12p40, IL-2 = interleukin-2, IL-4 = interleukin-4, IL-6 = interleukin-6, IL-8 = interleukin-8, INF-γ = interferon-gamma, LRV = low dose RV, MIP-1α = macrophage inflammatory protein-1alpha, MRV = middle dose RV, OHI-S = oral hygiene index-simplified, PPD = probing pocket depth, RV = resveratrol, TNF-α = tumor necrosis factor-alpha.

Keywords: endotoxin, inflammatory markers, periodontitis, resveratrol

1. Introduction

Periodontal disease is an oral inflammatory disease and characterized by chronic inflammation and local infection in periodontal tissues, which may lead to destruction of alveolar bone and/or tooth loss.[1] Pathologically, periodontal disease is caused by bacteria in dental biofilms that triggers inflammatory responses and further causes loss of both soft and hard tissue

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The present study was approved by the Ethics Committee of Mudanjiang Medical University.

Written informed consent for the publication was obtained from all patients.

The authors have no conflicts of interest to disclose.

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structures supporting teeth.[2] Periodontal pathogens with microbial biofilms and the host defenses result in systemic and/or local immune and inflammatory responses.[3] In addition, chronic inflammation of periodontal disease increases the risk factor of cardiovascular disease, chronic kidney disease and diabetes mellitus.[4] Furthermore, there are growing evidences that systemic inflammation in patients with periodontitis can predispose to various systemic diseases.[5] Moreover, the gingival crevicular fluid (GCF) is the principal target of bacteria and lead to accumulation of inflammatory factors (interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-1alpha (IL-1β), and interferon-gamma (INF-γ) etc) and bacterial endotoxin in the GCF in periodontal disease patients.[6]

Resveratrol (RV, 3,5,4′-trihydroxy-trans-stilbene), a polyphenol present in various foods, has been regarded as a promising drug in the prevention and treatment of inflammatory diseases.[7] Beneficial effects of RV are strongly related to the anti-inflammatory activation by inhibition of oxidative damage and mitochondrial dysfunction in cells.[8] RV treatment can improve periodontal disease progression by controlling inflammation.[9] RV also has anti-biofilm and anti-bacterial activity by targeting inflammatory and adhesive markers.[10] RV inhibits Porphyromonas gingivalis-induced inflammatory responses in human gingival fibroblasts and animal modeling of ligature-induced periodontitis.[11] At present, it demonists that RV may be beneficial as adjuvant therapy for patients with chronic periodontitis.[12]

The purpose of this study was to investigate the therapeutic effect of RV in patients with periodontitis. The periodontal status of patients with periodontitis was also compared between RV and placebo groups by using clinical attachment level (CAL), bleeding index (BI), oral hygiene index-simplified (OHI-S), and probing pocket depth (PPD). Safety assessments of RV were also investigated in patients with periodontitis.

2. Materials and methods

2.1. Study design

This placebo-controlled trial was conducted in Mudanjiang Medical University (Mudanjiang, China) between June 2017 and October 2018. A total of 190 patients with periodontitis were voluntarily enrolled in this study. Inclusion criteria: (1) age more than 18 years old; (2) patients diagnosed with periodontitis. Exclusion criteria: (1) smokers; (2) pregnant/lactating women; (3) alcoholics; (4) cancer patients; (5) systemic diseases patients; (6) patients received other treatments including blood thinner, Vitamin E, anti-inflammatory, or antibiotic medication etc. Finally, a total of 160 patients met inclusion criteria and allocated to intervention. Information on intervention allocation was placed in opaque envelopes and patients randomly selected one envelope. The selected patients were randomly divided into drug (RV, n = 120) and placebo (capsules, n = 40) groups. Drug groups included high-dose (500 mg/d) of RV (HRV, n = 40), middle-dose (250 mg/d) of RV (MRV, n = 40) and low-dose (125 mg/d) of RV (LRV, n = 40) with orally administration. All patients received an 8-week treatment. Outcomes of all patients with aggressive periodontitis were obtained and randomly assessed by three periodontists in our hospital (not one of the authors). This study was approved by the Ethical Committee of Mudanjiang Medical University. All patients signed written informed consent. All patients did not receive other drug therapy during the investigation.

2.2. Inflammatory markers analysis

A total of 5 ml of blood was collected in the morning hours from each patient. Inflammatory markers levels were detected using fluorescence detection kits (Milliplex 29-plex cyto-chemokine detection kits, Millipore, St. Charles, MO, USA) according to the manufacturer's instructions.

2.3. Analysis of levels of C-reactive protein (CRP) and fibrinogen

A total of 5 ml of blood was collected in the morning hours from patients in four groups using a 5 ml syringe. Serum and plasma were extracted from the blood by centrifuging at 3000× g for 10 minutes. Levels of CRP and fibrinogen were assayed using enzyme-linked immuno sorbent assay Kit (The EiAsy Way, Diagnostics Biochem Canada Inc).

2.4. Outcomes

Periodontitis was recorded by using CAL), bleeding index (BI), oral hygiene index-simplified (OHI-S), and PPD were analyzed as described previously.[13]

2.5. Safety and pharmacokinetics assessments

Safety of participants received RV or placebo treatment was evaluated in this study. Adverse events in patients received RV was scheduled using An independent Data and Safety Monitoring Board reviewed data quarterly as described previously.[14] All participants enrolled in this study for 24-hour pharmacokinetics of RV in plasma. These participants completed 24-hour pharmacokinetics of RV at various dosage at the indicated times (0, 0.2, 0.33, 0.5, 0.67, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 18, 20, 22 and 24 hours).

2.6. Statistical analysis

Data are expressed as mean ± standard deviation or number (percent). Data were analyzed using SPSS Statistics 22.0 (IBM Corp.). Student's paired *t* test and ANOVA with Dunn's multiple were applied to compare the mean values of placebo and RV treatment. A P-value $< .05$ was considered statistically significant.

3. Results

3.1. Characteristic of patients with periodontitis

A total of 160 patients with periodontitis were enrolled in this study. Patients were randomly divided into four groups and patients received 500 mg/d of RV (HRV, n = 40), 250 mg/d of RV (MRV, n = 40), 125 mg/d of RV (LRV, n = 40) and placebo (n = 40) for 8 weeks in a randomized, placebo controlled, cross-over trial. Characteristic of patients with periodontitis are shown in Table 1. The periodontal status of patients with periodontitis, sex, age, serum inflammatory, local inflammatory markers and systemic endotoxin were included in patients in drug and placebo groups. No significant difference was observed among groups.

Table 1

Characteristic of patients with aggressive periodontitis.

Parameters	Placebo (n = 40)	LRV (n = 40)	MRV (n = 40)	HRV (n = 40)
Male/female	18/22	19/21	17/23	18/22
Age (years)	26.52 ± 4.56	27.57 ± 5.10	26.30 ± 5.45	25.35 ± 4.40
CAL	5.94 ± 0.40	5.92 ± 0.52	5.88 ± 0.48	5.98 ± 0.50
BI	85.20 ± 8.20	86.52 ± 7.62	84.85 ± 8.08	86.85 ± 7.80
OHI-S	4.15 ± 0.52	4.33 ± 0.65	4.26 ± 0.47	4.28 ± 0.58
PPD	5.74 ± 0.50	5.82 ± 0.62	5.85 ± 0.60	5.80 ± 0.55
BMI (kg/m^2)	23.6 ± 2.5	23.2 ± 3.2	24.0 ± 3.0	24.4 ± 3.4
% sites > 4 mm	20.21 ± 6.20	21.62 ± 7.12	20.88 ± 6.64	21.52 ± 7.08
% sites > 7 mm	5.25 ± 1.05	5.40 ± 1.16	5.38 ± 1.20	5.45 ± 1.30
% Bleeding	17.30 ± 5.62	17.12 ± 4.85	16.86 ± 5.10	17.24 ± 5.20
% Plaque	42.17 ± 5.52	40.95 ± 5.42	41.65 ± 6.06	41.89 ± 6.18
Systemic endotoxin	2.85 ± 0.60	2.92 ± 0.64	2.80 ± 0.57	2.80 ± 0.50

Data are expressed as mean ± SD and analyzed using ANOVA with Dunn's multiple comparisons.

BI = bleeding index, BMI = body mass index, CAL = clinical attachment level, HRV = high dose RV, LRV = low dose RV, MRV = middle dose RV, OHI-S = oral hygiene index-simplified, PPD = probing pocket depth,

3.2. Effect of RV on the periodontal status of patients with periodontitis

The periodontal status was recorded in patients with periodontitis after 8-week treatment. As shown in Table 2, RV treatment significantly improved the periodontal status CAL, BI OHI-S and PPD compared to placebo in patients with periodontitis ($P < .01$). At the 8-week follow-up, the changes CAL, BI OHI-S and PPD were statistically significant for the comparison among 500 mg/d of RV and 250 mg/d of RV compared to 125 mg/d of RV group ($P < .05$). However, the CAL, BI OHI-S and PPD change did not achieve a clinically meaningful difference between patients with periodontitis received 500 mg/d of RV and 250 mg/d of RV ($P > .05$).

3.3. Effect of RV on systemic inflammation markers in patients with periodontitis

Several inflammatory markers in serum were evaluated in patients with periodontitis after 8-week RV or placebo treatment (Table 3). Outcomes showed that RV treatment significantly decreased serum levels of tumor necrosis factor-alpha (TNF-α), granulocyte-macrophage colony stimulating factor (GMCSF), macrophage inflammatory protein-1alpha (MIP-1α), fibrinogen, IL-2, CRP, INF-γ, IL-1β, interleukin-8 (IL-8), interleukin-10 (IL-10) and interleukin-12p40 (IL-12p40) in patients with periodontitis compared to placebo ($P < .01$). A higher serum levels of MCP-1, interleukin-4 (IL-4) and IL-6 were observed in RV-treated patients with periodontitis that placebo-treated patients ($P < .01$). Data found that there were no significant differences among 500 mg/d of RV and 250 mg/d of RV and 125 mg/d of RV group in regulating systemic inflammation markers in patients with periodontitis ($P > .05$).

3.4. Effect of RV on local inflammatory markers and systemic endotoxin in patients with periodontitis

The concentrations of local inflammatory markers and systemic endotoxin in patients with periodontitis were compared between RV and placebo groups (Table 4). Data found that concentrations of TNF-α, GMCSF, MIP-1α, fibrinogen, IL-2, CRP, INF-γ, IL-1β, IL-8, IL-10 and IL-12p40 were decreased by RV in diseased sites in patients with periodontitis compared to those patients received placebo ($P < .01$). Consistently, systemic endotoxin level was significantly decreased by RV treatment in patients with periodontitis compared to placebo ($P < .01$). Notably, 500 mg/d of RV showed significantly difference in decreasing systemic endotoxin in patients with periodontitis compared to 250 mg/d of RV and 125 mg/d of RV groups ($P < .05$). Reversely, RV treatment increased concentrations of MCP1, IL-6 and IL-4 in diseased sites in patients with periodontitis compared to placebo ($P < .01$).

3.5. Safety and tolerability

No significant differences of adverse events were observed between the RV and placebo-treated patients. The most common

Table 2

Effect of RV on the periodontal status of patients with aggressive periodontitis.

Parameters	Placebo (n = 40)	LRV (n = 40)	MRV (n = 40)	HRV (n = 40)
CAL	6.06 ± 0.37	4.18 ± 0.75**	3.76 ± 0.88$^{**\#}$	3.15 ± 0.96$^{**\#}$
BI	82.32 ± 7.80	47.20 ± 5.44**	40.35 ± 6.12$^{**\#}$	37.63 ± 7.20$^{**\#}$
OHI-S	4.40 ± 0.63	2.82 ± 0.52**	1.97 ± 0.45$^{**\#}$	1.72 ± 0.50$^{**\#}$
PPD	5.74 ± 0.50	2.82 ± 0.62**	2.15 ± 0.42$^{**\#}$	2.00 ± 0.40$^{**\#}$

Data are expressed as mean ± SD and analyzed using ANOVA with Dunn's multiple comparisons.

BI = bleeding index, CAL = clinical attachment level, HRV = high dose RV, LRV = low dose RV, MRV = middle dose RV, OHI-S = oral hygiene index-simplified, PPD = probing pocket depth, $^{**}P < .01$ vs. placebo. $^{\#}P < .05$ vs. LRV.

Table 3

Effect of RV on systemic inflammation markers in patients with aggressive periodontitis.

Parameters (pg/ml)	Placebo (n = 40)	LRV (n = 40)	MRV (n = 40)	HRV (n = 40)
TNF-α	625 ± 210	112 ± 102**	108 ± 118**	104 ± 98**
GMCSF	762 ± 120	270 ± 121**	256 ± 108**#	246 ± 110**
MIP-1α	632 ± 168	183 ± 68**	172 ± 75**	165 ± 60**
Fibrinogen	354 ± 124	124 ± 65**	118 ± 54**	110 ± 48**
IL-2	878 ± 168	242 ± 88**	220 ± 94**	212 ± 84**
CRP	3028 ± 425	1326 ± 324**	1245 ± 310**	1222 ± 286**
INF-γ	375 ± 132	168 ± 78**	160 ± 80**	156 ± 65**
IL-1β	543 ± 150	220 ± 105**	210 ± 90**	196 ± 68**
IL-8	756 ± 210	354 ± 142**	350 ± 152**	326 ± 137**
IL-10	430 ± 162	186 ± 54**	350 ± 152**	326 ± 137**
IL-12p40	518 ± 125	225 ± 108**	216 ± 114**	204 ± 108**
MCP-1	334 ± 118	538 ± 127**	552 ± 140**	567 ± 134**
IL-4	246 ± 95	414 ± 146**	454 ± 150**	470 ± 160**
IL-6	285 ± 100	456 ± 142**	486 ± 162**	493 ± 172**

Data are expressed as mean ± SD and analyzed using ANOVA with Dunn's multiple comparisons. **$P < .01$ vs. placebo.

CRP = C-reactive protein, GMCSF = granulocyte-macrophage colony stimulating factor, HRV = high dose RV, IL-1β = interleukin-1alpha, IL-2 = interleukin-2, IL-10 = interleukin-10, IL-12p40 = interleukin-12p40, MCP-1 = monocyte chemoattractant protein-1, IL-4 = interleukin-4, IL-6 = interleukin-6, IL-8 = interleukin-8, LRV = low dose RV, INF-γ = interferon-gamma, MIP-1α = macrophage inflammatory protein-1alpha, MRV = middle dose RV, TNF-α = tumor necrosis factor-alpha.

Table 4

Effect of RV on local inflammatory markers and systemic endotoxin in patients with aggressive periodontitis.

Parameters (pg/ml)	Placebo (n = 40)	LRV (n = 40)	MRV (n = 40)	HRV (n = 40)
TNF-α	415 ± 101	174 ± 96**	124 ± 118**	104 ± 98**
GMCSF	510 ± 205	266 ± 121**	240 ± 99**#	232 ± 92**
MIP-1α	444 ± 122	175 ± 78**	170 ± 70**	155 ± 53**
Fibrinogen	410 ± 130	202 ± 85**	189 ± 80**	182 ± 81**
IL-2	506 ± 227	322 ± 105**	310 ± 110**	297 ± 97**
CRP	878 ± 185	340 ± 132**	320 ± 140**	307 ± 126**
INF-γ	296 ± 45	152 ± 38**	140 ± 43**	127 ± 40**
IL-1β	408 ± 144	175 ± 80**	172 ± 76**	163 ± 55**
IL-8	356 ± 116	142 ± 70**	128 ± 67**	114 ± 43**
IL-10	246 ± 135	107 ± 38**	105 ± 52**	101 ± 46**
IL-12p40	422 ± 138	182 ± 96**	168 ± 77**	158 ± 60**
MCP-1	426 ± 129	655 ± 154**	668 ± 172**	685 ± 180**
IL-4	136 ± 42	360 ± 124**	377 ± 144**	386 ± 152**
IL-6	342 ± 105	610 ± 179**	635 ± 198**	550 ± 202**
Endotoxin (EU)	2.86 ± 0.72	1.04 ± 0.35**	0.69 ± 0.20**#	0.63 ± 0.22**#

Data are expressed as mean ± SD and analyzed using ANOVA with Dunn's multiple comparisons. **$P < .01$ vs. placebo, #$P < .05$ vs. LRV.

CRP = C-reactive protein, GMCSF = granulocyte-macrophage colony stimulating factor, HRV = high dose RV, IL-1β = interleukin-1alpha, IL-2 = interleukin-2, IL-10 = interleukin-10, IL-12p40 = interleukin-12p40, IL-4 = interleukin-4, IL-6 = interleukin-6, IL-8 = interleukin-8, LRV = low dose RV, INF-γ = interferon-gamma, MIP-1α = macrophage inflammatory protein-1alpha, MRV = middle dose RV, TNF-α = tumor necrosis factor-alpha.

Table 5

Safety and tolerability of RV in patients with aggressive periodontitis.

Adverse events	HRV	MRV	LRV
Nausea	6 (15%)	8 (20%)	8 (20%)
Diarrhea	9 (22.5%)	10 (25%)	11 (27.5%)
Hypotension	1 (2.5%)	1 (2.5%)	2 (5%)
Proteinuria	2 (5%)	3 (7.5%)	3 (7.5%)

Data are expressed as n (%).

HRV = high dose RV, LRV = low dose RV, MRV = middle dose RV.

adverse events were nausea and diarrhea in patients with periodontitis (Table 5). Findings observed that high-dose oral RV did not increase adverse events compared middle- and low-dose oral RV in patients with periodontitis. A total of 34 had nausea and diarrhea (14 HRV, 10 MRV, 10 LRV) in RV-treated group. A total of 3 patients occurred nausea and diarrhea in placebo-treated group.

3.6. Pharmacokinetics

Metabolism of RV was analyzed in patients with periodontitis in a 24-hour pharmacokinetic analysis (Table 6). Data found that the maximal plasma concentrations (C_{max}) of RV were 92, 52

Table 6

Pharmacokinetics of RV in patients with aggressive periodontitis.

Pharmacokinetics	C_{max}, plasma (ng/ml)	t_{max}, plasma (min)	Calculated $t_{1/2}$, plasma
HRV	92 ± 8	90 ± 4	842 ± 12
MRV	52 ± 6		
LRV	25 ± 5		

Data are expressed as mean ± SD.
HRV = high dose RV, LRV = low dose RV, MRV = middle dose RV.

and 25 ng/ml in HRV, MRV and LRV, respectively, in patients with periodontitis. The t_{max} of RV were 90 in patients with periodontitis. The half-life of RV was 842 minutes in patients with periodontitis.

4. Discussion

RV treatment possesses significant antimicrobial properties on periodontal pathogens by inhibiting vascular permeability.[15] RV may become a simple and inexpensive therapeutic strategy for treating periodontal disease by inhibiting the expression of virulence factorsfimbriae and gingipain.[10] Although previous clinical studies reported that systemic treatment with resveratrol reduces the progression and improving periodontal status of patients periodontitis,[12,16] this study not only investigated the anti-inflammatory and anti-endotoxic effect of RV, but also analyzed the pharmacokinetics of RV in patients with periodontitis. This study first reported the therapeutic effect of RV and analyzed the adverse events in patients with periodontitis. Outcomes showed that symptoms of periodontitis, local inflammatory markers and systemic endotoxin were significantly improved by RV treatment in patients with periodontitis. Notably, data found that high-dose oral RV is safe and well-tolerated in the treatment of periodontitis.

Inflammation is a physiological response to an injury or infection in disease sites in periodontitis, which may be a therapeutic target of periodontitis.[17] Detection and identification of inflammation in the GCF of diseased periodontal sites play important role in analyzing the disease mechanisms of periodontitis.[18] L.M. Shaddox *et al* showed patients with periodontitis presented higher levels of TNF-α, INF-γ, IL-1β, IL-2, IL-10, IL-12p40, GMCSF, and MIP-1α when compared with levels in their own healthy sites.[6] Data in this study found that RV treatment decreased systemic and local inflammatory markers TNF-α, GMCSF, MIP-1α, fibrinogen, IL-2, CRP, INF-γ, IL-1β, IL-8, IL-10 and IL-12p40 compared to placebo in patients with periodontitis (Table 3 and 4). Levels of MIP-1α and IL-6 are higher in localized periodontitis in patients with periodontitis than healthy controls.[19] IL-6 is found to be increased in GCF and gingival tissues of patients with periodontal disease.[20] The level of IL-4 is decreased in the patients with chronic periodontitis compared to those of healthy gingival samples.[21] Outcomes in this study not only found that RV increased concentrations of MIP-1α and IL-6, but also increased levels of IL-4 in serum and diseased sites in patients with periodontitis compared to placebo. This may explain the anti-inflammatory capacity of RV for patients with periodontitis, which may further lead to reestablish homeostasis. These data suggested that decreasing of systemic and local inflammatory markers production provided insight into the potential solution to preserve the inflammation in diseased sites in patients with periodontitis.

Elevated systemic level of endotoxin generated by bacterial is observed in localized periodontitis.[22] According to a recent study, RV prevents alveolar bone loss by attenuating the production of inflammation-related proteins, the formation of osteoclasts, and the production of circulating reactive oxygen species in an experimental rat model of periodontitis.[23] RV inhibits lipopolysaccharide-mediated cellular damages in human-originated gingival fibroblasts and also supports the potential of RV to suppress periodontitis-mediated tissue damages, which may improve a clinical approach of using of RV on human.[23] Therefore, treatment of teeth with periodontitis using different methods can decrease the level of endotoxin.[24] For successful 8-week maintenance therapy, RV significantly decreased systemic endotoxin level in patients with periodontitis compared to placebo (Table 4). Although patients with periodontitis show an elevated systemic level of endotoxin,[25] no studies report the residual endotoxins after receiving RV treatment. This study first found that reducing systemic endotoxins level was compatible with periradicular tissue healing. Therefore, RV can be one of important treatment methods for patients with periodontitis.

Our results showed that 500 mg/d of RV showed significantly difference in decreasing systemic endotoxin in patients with periodontitis compared to 250 mg/d of RV and 125 mg/d of RV groups. In addition, our data showed that RV treatment significantly improved the periodontal status CAL, BI OHI-S and PPD compared to placebo in patients with periodontitis after 8-week follow-up (Table 2). Furthermore, 500 mg/d of RV presented the best values of CAL, BI, OHI-S and PPD among various dose of RV (Table 2). Moreover, findings observed that 500 mg/d of RV was safe, and patients did not have additional adverse events in patients with periodontitis (Table 5). Notably, pharmacokinetics analysis found that RV maintained a certain half-life and could be metabolized within 24 h in patients with periodontitis (Table 6). Therefore, RV may be a safe and effective drug for the treatment of patients with periodontitis.

This study has several limitations. First, there was no direct comparison between RV and other drugs in the improvement of plaque reduction and gingivitis reduction in this study. Second, the associations among inflammation, endotoxin and degree of periodontitis did not analyze in this study. Third, long-term investigation did not perform in participants to monitor the effects of RV in patients with periodontitis. Despite these limitations, up to our knowledge, this is the first study to evaluate the efficacy and tolerability as well as the effectiveness of RV as a therapy in patients with periodontitis. In addition, this study evaluated the anti-inflammatory and anti-endotoxic effect of RV in patients with periodontitis. Furthermore, the pharmacokinetics of RV was also analyzed in patients with periodontitis.

In conclusion, findings in the current study support prescribing RV to improve periodontitis patients with periodontitis. Outcomes demonstrate that RV treatment has anti-inflammatory capacity and decreases systemic endotoxin in patients with periodontitis. Further studies are required to investigate the therapeutic effect of RV a large populations patient with periodontitis.

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Author contributions

QZ performed the experiments. SX, WX, YZ, and HL analyzed the data. DW designed the study and wrote the manuscript. All authors read and approved the final manuscript.
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