Radical-scavenging and Pro-/anti-inflammatory Activity of Tetracycline and Related Phenolic Compounds With or Without Visible Light Irradiation

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Abstract. Background/Aim: Microbial tetracycline (TC) pastes have been employed to treat oral bacterial infection. In the present study, we investigated the kinetic radical-scavenging and pro-/anti-inflammatory activity of TC with or without visible light irradiation (VLI). Materials and Methods: The radical-scavenging activity of TC and minocycline (MC) was determined by differential scanning calorimetry (DSC). The stoichiometric factor (n) and the rate constant of inhibition and propagation (k_{inh}/k_{p}) were determined. The levels of cyclooxygenase-2 (Cox2), tumor necrosis factor-α (Tnfα) or nitric oxide synthase 2 (Nos2) mRNA in RAW264.7 cells stimulated with lipopolysaccharide (LPS) were investigated using real-time reverse transcriptase-polymerase chain reaction. Results: The n and k_{inh}/k_{p} values for 1 mM TC in 2,2'-azobisisobutyronitrile and benzoyl peroxide systems were 0.1-0.2 and 119-250, respectively, whereas the corresponding values for quercetin (QU) and resveratrol (RE) were 2-4 and 7-15, respectively. In RAW264.7 cells stimulated with LPS, Cox2 and Tnfα mRNA were over-expressed in the presence of TC. MC down-regulated only the expression of Cox2 by about 50% in LPS-stimulated cells. The anti-inflammatory activity determined on the basis of Cox2 inhibition declined in the order QU>RE>MC>TC. Upon application of VLI, only TC down-regulated the expression of LPS-stimulated Cox2 and Tnfα mRNA. After exposure to VLI, TC, but not MC, markedly up-regulated hemoxygenase-1 (Ho-1) expression. Conclusion: TC is a chain-breaking antioxidant with a large k_{ph}. Upon activation by VLI, TC may undergo degradation and its degradation products affect pleiotropic mediators such as Cox2, Tnfα and Ho-1. TC may be useful as a local photodynamic therapy for periodontal diseases.

Tetracyclines such as tetracycline (TC) and minocycline (MC) are highly effective against gram-positive and gram-negative bacteria. These antibiotics are complex molecules with unique chemically connected ring systems (designated A through D) with multiple ionizable functional groups (Figure 1). Because tetracyclines possess multiple O- and N-functions, their strong tendency to form complexes with metals such as Cu^{2+}, Fe^{2+} and Zn^{2+} is well documented (1, 2). Also, previous studies have shown that TC may undergo degradation depending on pH, redox status and light conditions (3, 4). Many reports have indicated that tetracyclines have anti-protease effects such as matrix-metalloprotease (MMP) activity, anti-apoptotic effects, reactive oxygen species (ROS) scavenging activity and anti-inflammatory activity (1, 5-7). Tetracyclines have been used for the treatment of generalized and localized aggressive periodontitis that is refractory to treatment (8). However, with the emergence of resistant species of bacteria, tetracyclines have been replaced by more effective combinations of antibiotics (9). Additionally, tetracyclines have adverse effects such as transient retardation of bone growth, photosensitivity, permanent discoloration of developing teeth and teratogenesis, as well as hepatic and renal toxicity in susceptible individuals (10). They also have beneficial actions, including ROS scavenging and anti-inflammatory function (11).

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cytotoxicity and potential anti-inflammatory activity of various phytophenols on RAW264.7 cells stimulated with *Porphyromonas gingivalis* (PG) lipopolysaccharide (LPS) and fimbriae, and found that phytophenols such as curcumin, resveratrol (RE) and quercetin (QU) exhibited good anti-inflammatory activity (13-15).

In the present study, we investigated the kinetic antioxidant activity of TC and related phenolic compounds in terms of induction period (IP) and propagation rate constant (Rp) values using differential scanning calorimetry (DSC) to monitor the polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of azobisisobutyronitrile (AIBN) and benzoyl peroxide (BPO) under nearly anaerobic conditions (16, 17). The stoichiometric factor \((n)\), the number of free radicals trapped by 1 mole of antioxidant) and the ratio of the rate constant of inhibition \((k_{inh})\) and propagation \((k_{p})\), \(k_{inh}/k_{p}\) of these compounds were determined. Our quantitative model allowed rationalization because it is based on kinetic and thermodynamic data under nearly anaerobic conditions. Details of the mechanisms responsible for the antioxidant and pro-/anti-inflammatory activity of tetracyclines have not yet been fully clarified. TC and MC may inhibit the levels of proinflammatory cytokines, ROS, nitric oxide synthase (NO) and matrix metalloprotease (MMP) in RAW264.7 cells. We also investigated the inhibitory effects of TC, MC, QU and RE and their combinations with or without visible light irradiation (VLI) on the expression of *Cox2*, *Tnfa*, *Nos2* or *Ho-1* mRNA in LPS-stimulated RAW264.7 cells. Recent studies have reported that QU and other phytophenols are effective for the treatment of periodontitis (18). *In vitro* and animal studies have also shown that QU has potential antimicrobial activity, and can reduce the levels of inflammatory markers and cholesterol, as well as inhibit bone loss (19, 20). Therefore, we also investigated the radical-scavenging and pro-/anti-inflammatory activity of QU and RE as reference phenols for comparison with TC and MC.

**Materials and Methods**

**Materials.** TC, MC, QU were purchased from Tokyo Kasei Co. (Tokyo, Japan). trans-RE was obtained from Wako Pure Chemical Industries (Osaka, Japan). The chemical structures of these compounds are shown in Figure 1. Solutions of these compounds were prepared by dissolving each of them in dimethyl sulfoxide (DMSO), followed by dilution to the required concentrations using serum-free RPMI-1640 (Invitrogen Co., Carlsbad, CA, USA). Fetal bovine serum (FBS) was obtained from HyClone (Logan, UT, USA). *Porphyromonas gingivalis* (PG) ATCC33277 LPS was obtained from Wako Pure Chemical Industries.

**Cell culture.** The murine macrophage-like cell line RAW264.7, obtained from Dainippon Sumitomo Pharma Biomedical Co. Ltd. (Osaka, Japan), was used. The cells were cultured to a subconfluent state in RPMI-1640 medium supplemented with 10% FBS at 37°C and 5% CO₂ in air, washed, and then incubated overnight in serum-
Cox2, Nos2, Tnfα, and 18s rRNA and the PCR enzyme mix were diluted and used as a standard. TaqMan probes/primers were used for real-time PCR quantification was performed in triplicate using the GeneAmp Sequence Detection System 5700 software (Life Technologies, Japan) in accordance with the manufacturer’s instructions. The relative amount of target was calculated from standard curves generated for each PCR, and quantitative data with coefficients of variance of less than 10% were used for further analyses. Each calculated amount of mRNA was standardized by reference to that for 18s rRNA. Data were expressed as means of three independent experiments. Statistical analyses were performed using Student’s t-test and one-way ANOVA. Regression analysis was performed with StatMate III (ATMS Co., Ltd., Tokyo, Japan).

**Radical-scavenging activity.** The experiments to determine the antioxidant activity of carotenoids were performed using a DSC as reported previously (16, 22). Briefly, about 10 μl of experimental resin (MMA: 9.12-9.96 g) with 1.0 mol% AIBN or BPO and 0-0.5 mol% carotenoid were loaded into an aluminum sample container and sealed by applying pressure. The container was then placed in a DSC (model DSC 3100; Mac Science Co., Tokyo, Japan) kept at 70°C, and the thermal changes induced by polymerization were recorded for the appropriate time periods. The heat due to the polymerization of MMA was 13.0 kcal/mol. The conversion of all samples (%) was calculated from the DSC thermograms using the integrated heat evoked by MMA polymerization.

| System | Antioxidant | Con  | IP* | Max. peak | Time at | Rp** | Conversion |
|--------|-------------|------|-----|-----------|---------|-------|------------|
|        |             | mM   | min | mcal/min  | max. peak| min   | %/min      | %          |
| AIBN   | Control     | 0.1  | 3.59| 203.24    | 43.42   | 1.36  | 94.05      |
|        | Tetracycline| 0.1  | 4.21| 203.16    | 41.40   | 1.36  | 93.95      |
|        |             | 2    | 4.43| 189.29    | 42.93   | 1.30  | 94.77      |
|        |             | 4    | 4.95| 190.67    | 47.70   | 1.33  | 95.02      |
|        |             | 5    | 4.99| 196.49    | 43.73   | 1.39  | 95.21      |
|        | α-tocopherol | 1   | 8.28| 192.39    | 47.83   | 1.32  | 93.28      |
|        | Trolox      | 1    | 9.14| 189.54    | 49.35   | 1.37  | 93.95      |
|        | Quercetin   | 0.1  | 4.56| 199.74    | 41.47   | 1.39  | 93.01      |
|        |             | 1    | 9.90| 163.20    | 48.62   | 1.22  | 93.6       |
|        | Resveratrol | 0.1  | 4.87| 146.75    | 51.25   | 1.15  | 94.14      |
| BPO    | Control     | 0.1  | 7.20| 210.51    | 60.23   | 0.92  | 95.48      |
|        | Tetracycline| 0.1  | 7.54| 215.34    | 58.62   | 0.87  | 94.44      |
|        |             | 1    | 7.23| 217.18    | 59.55   | 0.89  | 95.95      |
|        |             | 2    | 7.72| 180.19    | 60.87   | 0.83  | 96.29      |
|        |             | 4    | 10.12| 174.29    | 64.93   | 0.90  | 94.5       |
|        |             | 5    | 10.40| 166.26    | 64.83   | 0.84  | 96.82      |
|        | α-tocopherol| 1    | 8.42| 195.54    | 60.60   | 0.85  | 93.06      |
|        | Trolox      | 1    | 7.89| 194.56    | 61.10   | 0.88  | 96.20      |
|        | Quercetin   | 0.1  | 10.38| 214.20    | 61.07   | 0.92  | 94.5       |
|        |             | 1    | 19.31| 164.86    | 72.18   | 0.81  | 94.42      |
|        | Resveratrol | 0.1  | 9.22| 209.47    | 59.72   | 0.92  | 94.34      |
|        |             | 1    | 25.67| 150.62    | 80.55   | 0.82  | 93.12      |

The values were determined based on the DSC data reported previously (23) using differential scanning calorimetry (DSC) monitoring of the polymerization of MMA (9.4 mol) initiated by thermal decomposition of AIBN (azobisisobutyronitrile) or BPO (benzoyl peroxide) under nearly anaerobic conditions at 70°C. The procedure is described in the text. Conversion, 84.9-96.9%. *Induction period, **Propagation rate constant. The values are the means for two or three independent experiments. Standard errors were <15%.
Table II. Antioxidant activities (stoichiometric factor, $n$; the rate constant of inhibition and propagation, $k_{inh}/k_p$) for tetracycline and related phenolic compounds using the induction period method in the 2,2’-azobis(isobutyronitrile, AIBN or benzoyl peroxide, BPO-methyl methacrylate, MMA polymerization system.

| Antioxidant | Con.(mM) | AIBN system | BPO system |
|-------------|----------|-------------|------------|
| Tetracycline |          | $n$         | $k_{inh}/k_p$ | $n$         | $k_{inh}/k_p$ |
| 0.1         | 0.3      | 817.4       | 0.5        | 391.7      |
| 1           | 0.2      | 118.9       | 0.1        | 249.3      |
| 2           | 0.1      | 91.4        | 0.1        | 231.6      |
| 4           | 0.1      | 55.3        | 0.2        | 38.0       |
| 5           | 0.1      | 54.6        | 0.1        | 37.1       |
| α-Tocopherol | 1        | 1.7         | 15.0       | 0.2        | 96.9       |
| Trolox      | 1        | 1.9         | 13.2       | 0.1        | 164.9      |
| Quercetin   | 0.1      | 3.3         | 74.0       | 2.7        | 34.2       |
|             | 1        | 1.8         | 15.0       | 4.1        | 10.3       |
| Resveratrol | 0.1      | 4.3         | 36.6       | 2.7        | 53.7       |
|             | 1        | 2.3         | 12.7       | 2.5        | 6.6        |

The values were calculated from the results of Table I using equations 1 and 2, respectively.

Measurement of the stoichiometric factor ($n$) and inhibition rate constant ($k_{inh}$). The $n$ value in equation 1 can be calculated from the induction period in the presence of inhibitors (antioxidants) as:

$$n = R_{IP}/[IH] \quad \text{(eq.1)}$$

where $IP$ is the induction period in the presence of a carotenoid inhibitor (IH). The number of moles of BPO or AIBN radicals trapped by the antioxidant was calculated with respect to 1 mol of the inhibitor moiety. The initiation rate ($R_I$) for AIBN and BPO at 70°C was $5.56 \times 10^{-6}$ mol $1^{-1}$sec$^{-1}$ and $2.28 \times 10^{-6}$ mol $1^{-1}$sec$^{-1}$, respectively, calculated on the basis of $n=2.0$ for 2,6-di-tert-butyl-4-methoxyphenol (16, 22, 23).

The $k_{inh}$ is given by

$$k_{inh} = \frac{[MMA]/[IP] \times [Rp]}{[IH]} \quad \text{(eq.2)}$$

where $k_p$ is the propagation rate constant, and MMA, IP and the rate of propagation ($Rp$) are defined above. A $k_p$ value of 9301 mol $1^{-1}$sec$^{-1}$ was used in this work (23).

Results

Radical-scavenging activity. First, we examined the kinetic radical-scavenging activity of TC in comparison with α-tocopherol (TOC), trolox, QU and RE. We used the induction period method in order to clarify the radical-scavenging mechanism of interaction between these phenol compounds and the AIBN (cyano-isopropyl, R+) and BPO (benzencarboxy, PhCOO+) radicals. The results are shown in Tables I and II.

Because there was a very small amount of air in the DSC sample container, induction time was also observed in the controls, since oxygen acts as an inhibitor and therefore, the IP value was determined as $IP_{sample}-IP_{control}$. The $n$ value for these compounds was calculated using eq. 1 and the $k_{inh}/k_p$ value was calculated using eq. 2. These results are shown in Table II. The $n$ value of 0.1-0.5 mM TC in the AIBN system was 0.1-0.3, whereas that in the BPO system was 0.1-0.5. Fully oxidized monophenol should have an $n$ value of 2, but the $n$ for TC was markedly less than 1. Therefore, TC was not a potent antioxidant. Also, the $k_{inh}/k_p$ value for 0.1-5 mM TC in the AIBN system was 54-817, whereas that in the BPO system was 37-392. The $k_{inh}/k_p$ value for TC increased as the concentration increased. In contrast, the $n$ value for 0.1 mM QU and RE in the AIBN system was 3.3 and 4.3, respectively, whereas that in the BPO system was 2.7 and 4.3, respectively. The $n$ value for 1 mM QU in the two systems was 1.8 and 4.1, respectively, whereas the corresponding value for RE was 2.3 and 2.5, respectively. The $n$ value of these compounds was 2-4. The $n$ value of fully oxidized RE and QU should be 4.

The $k_{inh}/k_p$ value for 0.1 mM QU and RE in the two systems was 34-74, whereas that for 1 mM was 7-15. The antioxidant activity of QU and RE was one order greater than that of TC. Interestingly, the $n$ and $k_{inh}/k_p$ values for TOC and trolox showed great difference between the AIBN and BPO systems. The $n$ value for these compounds in the AIBN system was about 2, whereas that in the BPO system was 0.1-0.2. Also, the $k_{inh}/k_p$ value for TOC and trolox in the BPO system was 97-165, whereas that in the AIBN system was 13-15. The lower antioxidant activities of TOC in the BPO system may be interpreted in terms of steric hindrance due to substituents of TOC, retarding the transfer of hydrogen to BPO (benzencarboxy, PhCOO+) radicals, and electronic effects that increase $k_{inh}$ by stabilization of the TOC phenoxyl radicals. Also, it can be interpreted by the fact that the
initiation rate (R_i) of the AIBN (cyano-isopropyl, R^+) radical derived from AIBN was about two-fold greater than that of the corresponding BPO (benzencarboxy, PhCOO^-) radicals. TOC and trolox possess higher intrinsic scavenging potency for AIBN radicals, resulting from their large n value. Our results suggested that these oxygen center radicals in biological systems may be scavenged less extensively by TOC (24). In the BPO system, the k_{inh}/k_p value at 1 mM
increased in the order RE (6.6)>QU (10.3)>TOC (96.9)>trolox (164.9)>TC (249.3). Also, the $k_{\text{inh}}$ value (1 mol$^{-1}$sec$^{-1}$) for TC, trolox, TOC, QU and RE declined in the order $2.3\times10^5>1.5\times10^5>9.0\times10^4>9.3\times10^3>6.1\times10^3$. The relationship between $k_{\text{inh}}/k_p$ and the square root of TC concentration ($C^{1/2}$) was investigated and a significant linear relationship was observed in the AIBN ($r^2=0.68$) and BPO ($r^2=0.96$) systems, respectively.

Figure 3. Inhibitory effects of minocycline in combination with resveratrol or quercetin on the expression of Cox2 (A), Tnfa (B) and Nos2 (C) mRNA in Porphyromonas gingivalis (PG)-LPS-stimulated RAW264.7 cells. The cells were pretreated for 30 min in 0.4 mM minocycline, 10 μM resveratrol or 10 μM quercetin, and 0.4 mM tetracycline with or without resveratrol or quercetin, and then incubated for 3.5 h with or without 100 ng/ml PG-LPS. Total RNAs were prepared, cDNAs were synthesized, and the expression levels of Cox2, Tnfa and Nos2 mRNA were determined by real-time polymerase chain reaction and standardized against the expression of 18s rRNA. The results are presented as means±standard error (SE) of three independent experiments, SE<15%. *Significantly different at p<0.01 vs. LPS group.
The $k_{inh}/k_p$ for TC in the BPO system is given by

$$k_{inh}/k_p = 457.1(\pm35.4) - 192.2(\pm22.9) \ C^{1/2} \ (n=5, \ p<0.01) \ (eq.3)$$

Unsaturated fatty acids are highly susceptible to lipid peroxidation. Hydrogen (H) of phenolic O-H groups can be abstracted by oxygen-centered radicals such as the peroxy (LOO$^\cdot$), alkoxy (LO$^\cdot$) and hydroxy (OH$^\cdot$) radicals; the
unsaturated region in the lipid chain can also react with singlet oxygen to form lipid peroxides. Eq. 3 suggested that TC may act as a chain-breaking radical scavenger, although its scavenging activity was very low.

Pro-/anti-inflammatory activity. Next, we investigated the anti-inflammatory activity of TC and related phenol compounds on LPS-stimulated RAW264.7 cells. Figures 2 and 3 show that LPS-stimulated Cox2 and Tnfa mRNA expression was upregulated by TC. Nos2 mRNA expression was inhibited by TC. MC strongly inhibited LPS-stimulated Cox2 and Tnfa mRNA expression. However, MC did not inhibit Nos2 mRNA expression. RE and QU markedly suppressed Cox2, Tnfa and Nos2 mRNA expression regardless of treatment with TC or MC. No synergistic activity was demonstrated between TC or MC and RE or
We also tested the influence of VLI on LPS-stimulated Cox-2 and Tnfα mRNA expression in the cells. Figure 4 shows that the TC-stimulated up-regulation of these mRNAs was markedly inhibited by VLI. The effect of MC was not affected by VLI. We further investigated whether the synergistic anti-inflammatory activity between TC and RE or QU affected LPS-stimulated Cox2, Tnfα, and Nos2 mRNA expression by VLI. Figures 5 and 6 show that the anti-inflammatory effects of TC and RE were enhanced by VLI, but that QU did not have an enhancing effect and showed pro-inflammatory properties. These observations suggested to us the necessity of investigation whether the degradation of TC may stimulate the production of secondary biological active products by VLI. Therefore, to clarify the mechanism responsible for the anti-inflammatory effect of VL-irradiated TC, we investigated whether VLI stimulated Ho-1 expression.

Figure 6. Effect of visible-light irradiation on Cox2 (A), Tnfα (B) and Nos2 (C) mRNA expression in RAW264.7 cells consecutively stimulated with tetracycline and quercetin in combination and Porphyromonas gingivalis (PG)-LPS. The cells were pretreated for 30 min in 0.4 mM tetracycline or 10 μM quercetin, or tetracycline with or without quercetin, and with or without exposure to visible-light irradiation for 3 s. They were then incubated for 3.5 h with or without 100 ng/ml PG-LPS, and total RNAs were prepared. cDNAs were synthesized, and the expression levels of Cox2, Tnfα and Nos2 mRNA were determined by real-time polymerase chain reaction and standardized against the expression of 18s rRNA. The results are presented as means±standard error (SE) of three independent experiments, SE<15%. *Significantly different at p<0.01 vs. LPS group.
in the same sample. Figures 7 and 8 show that stimulation of TC activated by VLI greatly up-regulated the expression of Ho-1 mRNA (approx. 4-fold). In contrast, except for TC activation by VLI, Ho-1 expression was not affected by activation of any of the other compounds tested by VLI.

**Discussion**

Oxidative stress caused by bacterial LPS, chemicals, light, redox, pH etc. has been implicated in the pathology of many disease and conditions including cardiovascular disease, periodontal diseases, diabetes, inflammatory conditions, cancer and aging. Antioxidants may reduce oxidative stress by scavenging free radicals and ROS, and inhibiting lipid peroxidation (25). Thus, the radical-scavenging activity of tetracycline and related phenols is a very important aspect of their biological activity. TCs are complex molecules with a unique chemically connected four-ring system with multiple ionizable functional groups (Figure 1). The dimethyl amino group at the C4 carbon, like that in MC, has been shown to be necessary for increased antioxidant activity. The antioxidant activity of tetracycline is probably lower than that of MC, since the phenolic O-H bond dissociation enthalpy (O-H-BDE) of aminophenols is lower than that of the parent phenol like TC. Cell-free assays have shown that the IC$_{50}$ of the antioxidant activity of MC is 3-40 μM, that is 200-300 times more potent than that of TC. The antioxidant activity of MC is comparable to that of TOC and trolox (26). However, no previous report has documented the kinetic radical-scavenging activity of TC. Our DSC studies have made it possible to examine the radical-scavenging activities of very small antioxidant compounds such as β-carotene and...
Anti-inflammatory activity. Interestingly, the expression of Cox2 mRNA expression slightly but significantly, whereas TC at 0.4 mM suppressed that of Nos2, indicating that these compounds exert strong anti-inflammatory activity. As shown by eq. 3, the antioxidant activity of TC was dependent on its concentration; as the concentration of TC increased, the $k_{inh}/k_p$ value decreased. The $k_{inh}/k_p$ value of 4 mM TC was similar to that of 0.1 mM QU or RE (Table II). Together with our previous reports, the present results indicated that in LPS-stimulated RAW264.7 cells, the approximately 50% effective dose in terms of Cox2 mRNA expression (ED$_{50}$, mM) increased in the order QU (0.01)>RE (0.03)>MC (0.4, this work)>TOC (0.5)>>TC (4, this work, data not shown) (14, 15, 31). The rank order of anti-inflammatory activity for the antioxidants tested may be comparable to that of their radical-scavenging activity. The anti-inflammatory activity of phenolic compounds was thought to be possibly related to their antioxidant capacity.

With regard to the VLI-mediated effects in LPS-stimulated cells, TC down-regulated the expression of Cox2 and Tnfα mRNAs by approximately 50%. TC may undergo degradation upon exposure to VLI. The TC degradation products activated by VLI may suppress Cox2 and Tnfα mRNA expression.

We also examined the synergistic effects of TC-related compounds and polyphenols on the anti-inflammatory action. Tetracycline compounds did not promote the anti-inflammatory action of polyphenols (Figure 2). However, RE had a synergistic effect with VLI on the anti-inflammatory action (Figure 5). RE is a stilbene derivative and causes a

![Figure 8. Minocycline does not induce Ho-1 mRNA expression upon exposure to visible-light irradiation in Porphyromonas gingivalis (PG)-LPS-stimulated RAW264.7 cells. The cells were pretreated for 30 min with 0.4 mM minocycline with or without exposure to visible-light irradiation for 3 s, and then incubated for 3.5 h with or without 100 ng/ml PG-LPS. Total RNAs were then prepared, cDNAs were synthesized, and the expression levels of Ho-1 mRNA were determined by real-time polymerase chain reaction and standardized against the expression of 18s rRNA. The results are presented as means±standard error (SE) of three independent experiments, SE <15%.](image-url)
cis-trans isomerization reaction when exposed to light irradiation. cis-RE has been reported to have antioxidant activity and to down-regulate some inflammatory genes in mouse macrophages (32). Therefore, the synergy of the anti-inflammatory effects of TC and RE upon exposure to VLI may be due to the action of cis-RE.

Our results suggested that VLI-activated TC may undergo degradation and that its degradation products may markedly enhance the expression of Ho-1. Using electron spin resonance and photometry, Chen et al. have found that \( \text{O}_2^\cdot \) and \( \text{H}_2\text{O}_2 \) (ROS) were generated in TC solution exposed to sunlight over the natural pH range, and that consequently, direct photodegradation of TC occurred (4). Several degradation products of TC have been reported, as is the case for stereoisomers of tetracycline (4-epitetracyclines), 4-epoxytetracycline, etc. (33). These degradation products resulting from VLI may possibly affect inflammatory mediators.

On the other hand, Ya et al. have reported that tetracyclines (TC, DC, MC) can accumulate in bacterial ribosomes, where they are photoactivated by blue light (BL) (415 nm) and ultraviolet light (UVA) (365 nm), resulting in microbial killing via ROS generation, and that no photobleaching of tetracyclines was observed upon exposure to either UVA or BL at <50 J/cm\(^2\) (34). Our present results indicated that MC, QU and RE were fairly photostable in RAW264.7 cells. HO-1, the inducible isoform of HO, catalyzes the degradation of heme into biliverdin, iron, and carbon monoxide (CO), and inhibits immune responses and inflammation in vivo. Biliverdin and bilirubin are potent antioxidants that attenuate oxidative stress (35), and HO-1 has anti-inflammatory, anti-oxidant, and anti-proliferative effects (36-38).

Transcription of Ho-1 is upregulated by many factors including heme, hydrogen peroxide, UV irradiation, hypoxia, and physical stress (39). On the other hand, since TC strongly induced Ho-1 expression upon exposure to VLI, TC degradation products may disrupt the inhibitory activity of Kelch-like ECH-associated protein 1 (Keap1) on NRF2, which is a major EpRE/ARE activating transcription factor. This may have subsequently triggered the EpRE/ARE system, resulting in upregulation of the Ho-1 gene (40, 41). TC, but not MC, was photo-activated by VLI and greatly upregulated the expression of Ho-1. It is considered that TC may induce HO-1-mediated antioxidative action upon exposure to VLI, leading to a cytoprotective action such as a strong anti-inflammatory effect (39). It has also been pointed out that a decrease in the expression and activity of Ho-1 may lead to an increase in inflammation (42). Therefore, it has been suggested that induction of Ho-1 expression by VLI may offer a new anti-inflammatory function to TC in many chronic inflammatory diseases. TC as a photosensitizer may be useful for local photodynamic therapy in patients with periodontal diseases that cause alveolar bone loss.

Conclusion

LPS-induced expression of Cox2, Nos2 and Tnfa in in RAW264.7 cells is suppressed by TC, MC, RE and QU. The anti-inflammatory activity declines in the order QC>RE >MC>TC. These compounds are chain-breaking antioxidants and the radical-scavenging effect determined by the IP method declines in the order QC>RE>MC (estimated)>TC. TC is a VLI-sensitive antibiotic. TC at low concentrations greatly up-regulates Ho-1 expression when activated by VLI. Further work is required to fully elucidate the underlying mechanism of the antioxidant and pro-/anti-inflammatory activities of TC.

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Authors’ Contributions

S. Fujisawa conceived the present study. Y. Murakami verified the analytical methods. All Authors discussed and contributed to the final manuscript.

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