Quebecol shows potential to alleviate periodontal tissue damage and promote bone formation in *in vitro* models

Sébastien Cardinal[1,*], Amel Ben Lagha[2], Jabrane Azelmat[2] and Daniel Grenier[2]

[1] Département de biologie, chimie et géographie, Université du Québec à Rimouski, 300 allée des Ursulines, Rimouski, QC, Canada, G5L 3A1.

[2] Groupe de recherche en écologie buccale, Faculté de médecine dentaire, Université Laval, 2420 rue de la Terrasse, Québec, QC, Canada, G1V 0A6.

[*] Corresponding author: Email: sebastien_cardinal@uqar.ca, Telephone : 418-723-1986 ext.1355.

Abstract

Quebecol is a polyphenolic compound initially isolated from Canadian maple syrup in 2011. Recently, our group demonstrated in a macrophage model that quebecol inhibits the secretion of pro-inflammatory cytokines and reduces the activation of the NF-κB transcription factor. In this study, we further explored the therapeutic potential of quebecol against periodontal disease, an inflammatory disorder of bacterial origin affecting the tooth-supporting tissues. More specifically, the effects of this natural compound on matrix metalloproteinase (MMP) activity and macrophage secretion, as well as on the mineralization activity of osteoblasts (bone-forming cells), were investigated. Results showed that exposing LPS-treated macrophages to quebecol led to a significant decrease in the secretion of MMP-8 and MMP-9. In addition, quebecol dose-dependently inhibited the catalytic activity of MMP-9. Quebecol also enhanced the mineralization activity of osteoblasts. This study brought forward additional evidence to support the potential of quebecol as a nutraceutical agent against periodontitis.

Introduction

Periodontitis, a destructive form of periodontal disease that causes permanent damage to the periodontium, is a widespread pathology that currently ranks as the sixth most common chronic inflammatory disorder in the world.[1] As such, it remains to this day a worldwide public health concern with major economic and social burdens. Periodontitis results from an uncontrolled inflammatory response of mucosal and immune cells to specific gram-negative strictly anaerobic bacterial species colonizing the subgingival sites as a biofilm.[2, 3] Evolution of this pathology leads to the progressive destruction of periodontal connective tissue and alveolar bone that may cause tooth loss.[1] This pathogenic process involves the expression and secretion by resident and infiltrating cells of the periodontium of a wide variety of pro-inflammatory mediators and matrix metalloproteinases (MMPs) that modulate periodontal tissue destruction and perpetuate the pathology.[4-6]

MMPs, a family of host-derived calcium-dependent zinc-containing proteolytic enzymes, are involved in the degradation of extracellular matrix (ECM) components, such as collagen, fibronectin, laminin, and elastin, during both normal and pathological conditions.[7] MMPs are produced by the major cell types found in periodontal tissue, including epithelial cells, fibroblasts, neutrophils, and macrophages.[5, 6] In healthy periodontal tissues their expression is quite low and their activity is regulated by endogenous molecular agents called tissue inhibitors of metalloproteinases (TIMPs).[5, 6] However, an over-expression of MMPs has been associated to the pathological tissue destruction process observed during periodontitis.[6, 8, 9] Therefore, the excessive production and activity of MMPs has been proposed as a potential target for pharmacological intervention in periodontitis.[5, 10, 11]

MMPs also markedly contribute to alveolar bone loss occurring during periodontitis, which is a process triggered by osteoclasts and attributed to the inflammatory nature of the disease.[12] Osteoblasts, which are cells involved in the process of bone formation, can restore the normal bone homeostasis.[13, 14] These cells are derived from mesenchymal cells and secrete the organic matrix (mostly collagen) and induce calcification during the

formation of new bones.[13, 14] Targeting osteoblast activity may therefore represent a relevant therapeutic strategy against periodontitis.

Quebecol (ethyl 2,3,3-tris(4-hydroxy-3-methoxyphenyl)propan-1-ol; compound **1**; Fig. 1a) is a polyphenolic compound initially isolated from Canadian maple syrup in 2011.[15] It is formed during the heating process required to transform the sap of *Acer saccharum* into syrup. *In vitro* bioassays brought evidence that quebecol may represent a promising chemopreventive or chemotherapeutic agent.[16-18] Recently, we developed an efficient synthesis protocol for quebecol (Fig. 1a)[19] and reported its *in vitro* anti-inflammatory activity.[20] More precisely, we showed that a treatment of lipopolysaccharide (LPS)-stimulated human macrophages with quebecol leads to an inhibition of the secretion of two pro-inflammatory cytokines (IL-6 and TNF-α), while a reduction of the activation of the NF-κB transcription factor was also observed.[20] In parallel to our work on quebecol itself, twenty related compounds (precursors, isomers, and substructures) were synthesized and used in structure-activity relationship studies.[20, 21] In brief, these studies showed that quebecol (Fig. 1a) and its diarylmethane substructure (compound **3**; Fig. 1b) exhibit anti-inflammatory activity.[20, 21] Results also demonstrated that the substitution pattern on the aromatic rings of these active structures (compounds **4** and **5**; Fig. 1b) led to a slightly enhanced activity but higher cytotoxicity.[21] It was concluded that the natural product **1** was still the most promising anti-inflammatory compound based on biological activity and toxicity arguments.

Encouraged by the above results, we wished to further explore the therapeutic potential of quebecol against periodontitis by focusing on the two specific therapeutic targets discussed above: MMPs and osteoblasts. First, we investigated the ability of quebecol to attenuate the secretion of MMPs by LPS-stimulated macrophages and to inhibit the catalytic activity of MMP-9. Second, we assessed the effects of quebecol on the mineralization activity of osteoblasts.

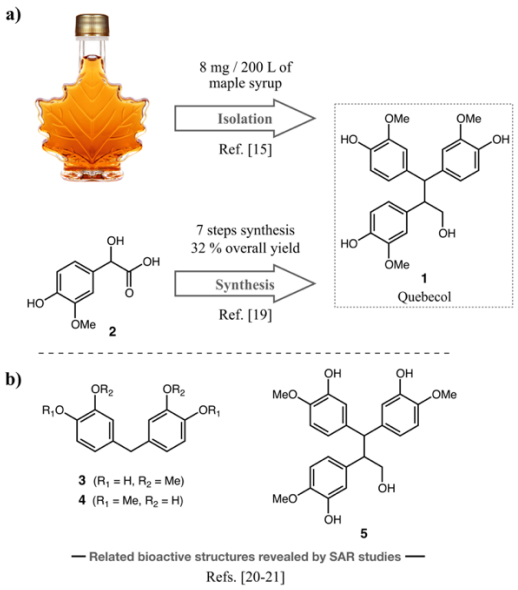

Figure 1. Quebecol and relevant related structures from our previous work. (a) Structure of quebecol and accessibility from isolation or synthesis; (b) Relevant bioactive structures identified in our previous structure activity relationship (SAR) studies.

Results

Quebecol and MMP secretion by macrophages

The effect of quebecol on the *in vitro* regulation of MMPs was investigated by assessing its capacity to impair their secretion in a macrophage model, which represents a leukocyte type recognized as an important producer of MMPs in the context of periodontitis. To this end, we assessed its effect on the basal and LPS-induced secretion of MMP-3, MMP-8, and MMP-9. These three specific MMPs were selected on the basis of their known association with active periodontitis.[5, 6, 8-10] It is also worth mentioning that these three MMPs were those secreted in the highest amount by the U937 macrophages used in this study.

The secretion of MMP-3, MMP-8, and MMP-9 by macrophages kept under normal conditions or stimulated with *A. actinomycetemcomitans* LPS is reported in Fig. 2. MMP-9 was found to be produced in significantly greater amounts than MMP-3 and MMP-8. The stimulation of macrophages with LPS did not significantly modify the secretion of MMP-

3 and MMP-9, while it increased the secretion of MMP-8 by 20.8% ($p < 0.001$). More specifically, LPS-stimulated macrophages secreted 650 ng/mL, 3 109 pg/mL, and 2 354 pg/mL of MMP-9, MMP-8 and MMP-3, respectively.

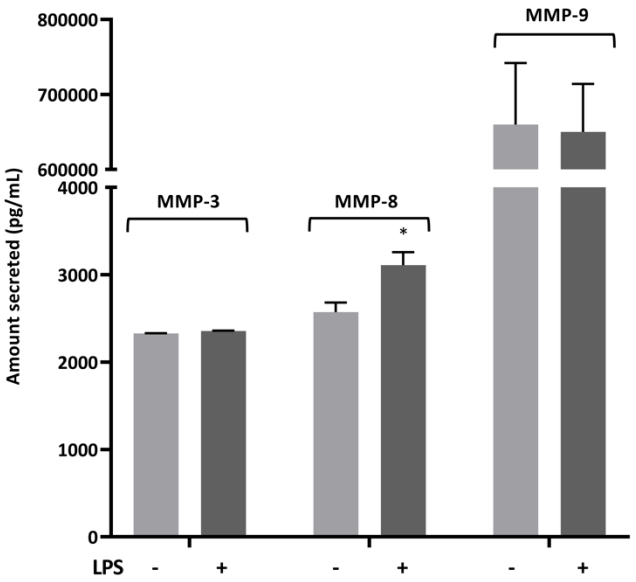

Figure 2. Effect of *A. actinomycetemcomitans* LPS on the secretion of MMP-3, MMP-8, and MMP-9 by macrophages. Means ± standard deviations of triplicate assays. *: $p < 0.001$.

We first investigated the effect of quebecol on MMP secretion by macrophages kept under normal conditions (without LPS stimulation). When the cells were exposed to the highest concentration (500 μM) of quebecol, the secretion of MMP-3, MMP-8, and MMP-9 significantly decreased by 15.9%, 34.4%, and 26.8% ($p < 0.001$) (Fig. 3). Quebecol at concentrations ≤ 125 μM did not reduce the secretion of any of the MMPs in a significant manner.

Then, the same analysis was performed using macrophages that were stimulated with *A. actinomycetemcomitans* LPS in a way to simulate an inflammatory condition. As shown in Fig. 3, while only a slight inhibition of MMP-3 secretion by quebecol was observed for LPS-stimulated macrophages, significant dose-dependent reductions in the secretion of MMP-8 and MMP-9 were caused by the presence of quebecol even at the lowest concentration tested (31.25 μM), with an inhibition of 15.2% and 21.4%, respectively. Complete tabulated results can be found in the electronic supplementary information.

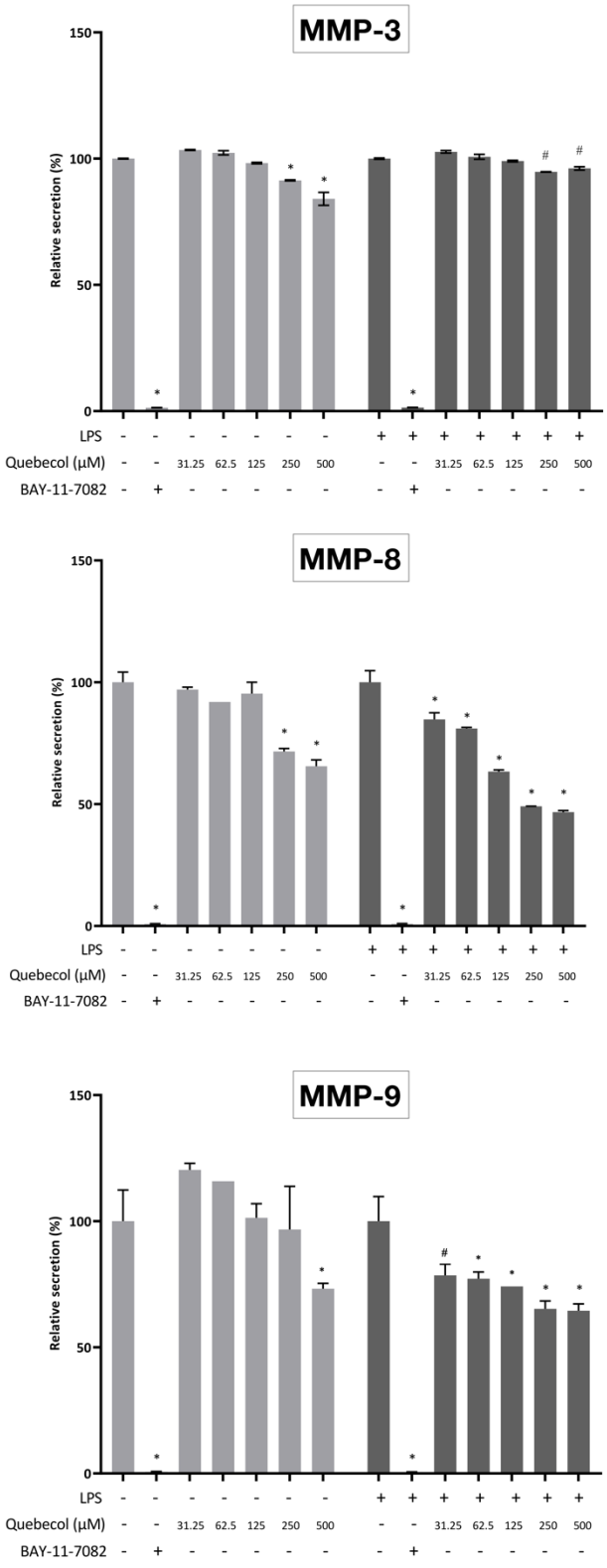

Figure 3. Inhibitory effect of quebecol on the secretion of MMP-3, MMP-8, and MMP-9 by macrophages stimulated with *A. actinomycetemcomitans* LPS or not. Means ± standard deviations of triplicate assays. *: $p < 0.001$, #: $p < 0.05$.

Overall, a difference regarding the inhibitory activity of quebecol on the secretion of MMP-8, MMP-9 and to a lesser extent MMP-3 by macrophages kept under normal conditions or stimulated with *A. actinomycetemcomitans* LPS was observed. Such a selectivity would be advantageous regarding a potential clinical use since this natural compound would have a minimal impact on healthy gingival tissues.

Quebecol and MMP-9 activity

Given the interesting results obtained regarding the effect of quebecol on MMP-9 secretion and that MMP-9 was produced in high amounts by macrophages, we selected this MMP to investigate the effect of quebecol on its catalytic activity.[22] As reported in Table 1, quebecol reduced the degradation of gelatin by MMP-9 in a dose-dependent manner. More specifically, the presence of quebecol at 250 and 500 μM inhibited the activity of MMP-9 by 42% and 92.6%, respectively. It is interesting to note that while the secretion of this MMP by macrophages stayed similar between these two concentrations of quebecol under an inflammatory context, an important difference with respect to MMP-9 activity was observed.

Table 1. Effect of quebecol on MMP-9 activity.

Concentration (μM)	Relative activity (%)
0	100 ± 2
62.5	92.2 ± 0.6
125	82 ±1*
250	58 ± 3*
500	7.4 ± 0.6*

*: Significantly different from control (no quebecol) at $p < 0.001$

Quebecol and osteoblast mineralization activity

An important process involving osteoblasts during bone formation is their mineralization activity. It was thus of interest to investigate the effect of quebecol on the mineralization activity of SaOS-2 cells by monitoring the formation of extracellular mineral deposits with a staining agent (Alizarin Red S) that selectively binds to calcium salts. As shown in Fig.

4, the presence of quebecol promoted the formation of calcium deposits that appear as red mineralized nodules. More specifically, quebecol, from a concentration of 12.5 µg/mL (corresponding to 29.3 µM), dose-dependently promoted the mineralization activity of osteoblasts (Fig. 5). A marked increase in mineralization activity was observed at the highest concentrations tested. For instance, the presence of quebecol at 100 µg/mL (corresponding to 234 µM) increased the mineralization activity by 2.4-fold.

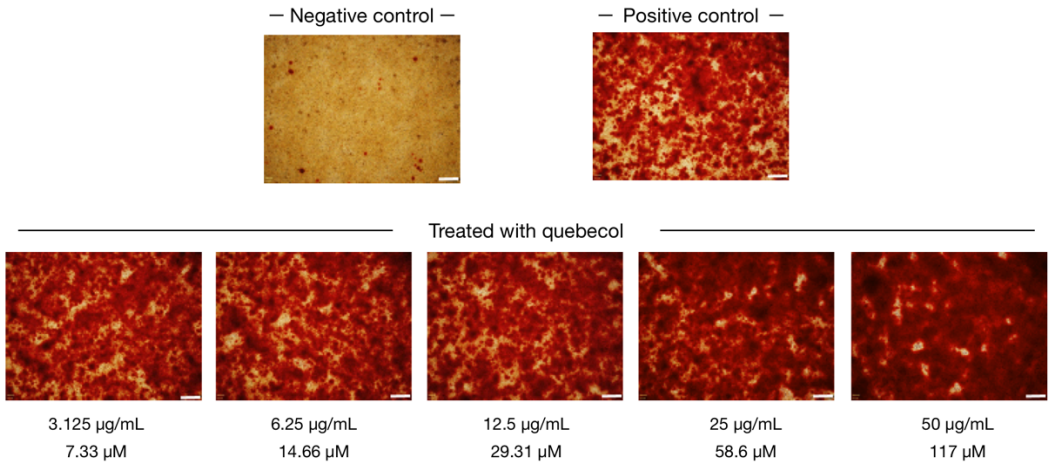

Figure 4. Effect of quebecol on the mineralization activity of SaOS cells. Representative microscopic images of Alizarin red staining of calcium deposits. Scale bar: 150 µm.

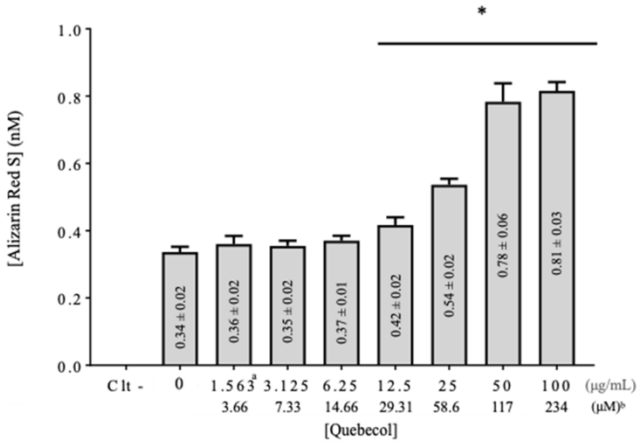

Figure 5. Effect of quebecol on the mineralization activity of SaOS cells as expressed by Alizared red concentrations. Means ± standard deviations of triplicate assays. *: $p < 0.001$.

Discussion

Polyphenols are compounds that possess an aromatic ring with at least one hydroxyl group and show high heterogeneity in their structure, from simple molecules to complex polymers.[23] Due to their antimicrobial, antioxidant, and immunomodulatory properties, there has been a growing research interest in polyphenols with respect to oral health benefits.[24-26] A process-derived polyphenolic compound isolated from maple syrup, named quebecol after the Canadian province of Quebec (the world's leading producer of maple-derived products), has been the topic of several studies.[15-21] Based on our recent investigations reporting the anti-inflammatory properties of quebecol,[20] the present study further investigated its potential as a prophylactic and therapeutic agent against periodontitis, a widespread chronic inflammatory disease of bacterial origin that affects the tooth-supporting tissue.

The continuous, high production of MMPs by infiltrating and resident cells of the periodontium triggered by periodontopathogens contributes to connective tissue destruction and alveolar bone resorption.[6, 8, 9] In fact, it is well known that MMP levels and activities are significantly higher in the gingival tissue and gingival crevicular fluid of periodontitis-affected individuals than from healthy subjects.[6, 8, 9] Therefore, MMP inhibitors may have interesting potential for use as an effective therapeutic approach for treating periodontitis patients.[10-11] Consequently, quebecol was tested for its effect on MMP secretion in a macrophage model, as macrophages are potent MMP producers and constitute an important part of the inflammatory infiltrate in active periodontal lesions.[27] In the present study, we showed that *A. actinomycetemcomitans* LPS acts as an inducer of the secretion of MMP-8 by macrophages, while it has no effect on the secretion of MMP-3 and MMP-9. Our results showed that quebecol caused a significant inhibition of the secretion of MMP-8 (50.9%) and MMP-9 (34.8%) when the cells were treated with this phenolic compound (250 μM) in an inflammatory context (stimulation with LPS). MMP-8 (collagenase-2) and MMP-9 (gelatinase B) are present in high concentrations in inflamed periodontitis sites and are strongly associated with the progression of periodontitis.[29] Periodontal therapy (scaling and root planing) has been shown to significantly reduce

salivary MMP-8 and MMP-9 levels,[30, 31] suggesting that these two MMPs are potential markers for monitoring periodontal disease activity. Consequently, the ability of quebecol to attenuate the secretion of these two MMPs may contribute to reduced periodontal tissue destruction. Although the exact inhibitory mechanism of action of quebecol was not investigated, it is likely related to the previously reported capacity of quebecol to impair NF-κB activation in macrophages.[20] Indeed, previous studies have shown that the expression of MMPs by macrophages involves the NF-κB signaling pathway.[32, 33] Moreover, La et al.[34] reported that cranberry proanthocyanidins reduce MMP-9 secretion in macrophages through the inhibition of NF-κB activation.

To complement the results of the impact of quebecol on MMP secretion by macrophages, we assessed its effect on MMP-9 activity. This analysis revealed that the polyphenolic compound exerts a marked dose-dependent inhibitory effect on the capacity of MMP-9 to degrade its natural substrate (gelatin). Although the mechanism of action remains to be elucidated, it can be hypothesized that quebecol, with its four phenol groups, acts as a ligand on MMP-9 that either deactivates the zinc ion on the catalytic cycle or blocks the access of substrate to the active site of the enzyme.

Since bone formation is instrumental to counterbalance tissue destruction in the context of periodontitis, we also studied the effect of quebecol in an *in vitro* osteoblast model. While they secrete collagen and proteins during bone formation, osteoblasts also trigger calcium and mineral deposition, leading to the formation of hydroxyapatite on the newly formed bone matrix.[13, 14] We demonstrated that the mineralization activity of osteoblasts was positively affected by quebecol in a dose-dependent fashion, the most important increase in the formation of extracellular calcium deposits being observed at the highest concentrations tested. It is also noteworthy to mention that results obtained in a parallel study led by our group strongly suggest that the enhanced mineralization activity caused by quebecol is not due to an effect of this compound on the population of osteoblast cells (see Supporting Information). In accordance with our results, polyphenols from green tea are also known to promote osteoblastic mineralization.[35]

In conclusion, when taken together, the results of our present and previous studies[20] point towards the potential use of quebecol as a promising multi-target therapeutic agent against periodontitis and, most likely, other inflammatory disorders. Indeed, at non-cytotoxic concentrations, quebecol was found to inhibit the secretion of MMP-8 and MMP-9 in a macrophage model (stimulated with LPS), to inhibit the catalytic activity of MMP-9, and to stimulate osteoblast mineralization activity, in addition to impairing NF-κB activation and IL-6 secretion in macrophages.[20] In this regard, studies on the benefits provided by the use of oral hygiene products (mouthrinses and chewing gums) or slow periodontal-release devices (to be inserted in diseased periodontal sites) containing quebecol are of high interest. Given these promising perspectives, we are currently exploring the effect of this natural polyphenol on periodontopathogenic bacteria, which represent the second contributing factor to periodontitis. Studies on the effect of quebecol on alveolar bone osteoclasts are also underway.

Methods

Synthesis of quebecol

Quebecol was synthesized and characterized according to a previously reported protocol.[19]

Preparation of monocyte-derived macrophages

U937 human monocytes (ATCC CRL-1593.2) were purchased from the American Type Culture Collection (Manassas, VA, USA) and were cultivated under the following conditions: RPMI-1640 + 10% heat-inactivated fetal bovine serum (FBS) + 100 µg/mL of penicillin G/streptomycin, 37°C, and 5% CO_2 atmosphere. In a way to induce their differentiation into adherent macrophage-like cells, the monocytes (2.5 x 10^5 cells/mL) were incubated for 48 h in RPMI-10% FBS medium containing 100 ng/mL of phorbol myristic acid (PMA, Sigma-Aldrich Canada Ltd, Oakville, ON, Canada), and then harvested by scraping. After centrifugation (1200 g for 5 min), the adherent macrophage-like cells were washed, suspended in RPMI-1% FBS (concentration of 1 x 10^6 cells/mL), and seeded into wells of 12-well microplates (1 x 10^6 cells/well) which were incubated for 24 h at 37°C in a 5% CO_2 atmosphere.

Determination of MMP-3, MMP-8, and MMP-9 secretion by macrophages

The aforementioned macrophages were treated with two-fold serial dilutions of quebecol (500 to 31.25 μM in culture medium) for 2 h prior to stimulation with LPS (1 μg/mL) prepared from *Aggregatibacter actinomycetemcomitans* ATCC 29522 according to a previously described protocol.[36] A series of macrophages treated with quebecol but not stimulated with LPS was also prepared. After a 24 h incubation (37°C, 5% CO_2 atmosphere), the culture supernatants were collected and stored at -20°C until analyzed. Cells incubated in culture medium without quebecol and/or LPS were used as negative controls. BAY-11-7082 (Selleckchem, Houston, TX, USA) at a concentration of 5 μg/mL was used as a positive inhibitory control. Quantification of MMP-3, MMP-8, and MMP-9 in cell culture supernatants was performed with enzyme-linked immunosorbent assay (ELISA) kits (R & D Systems, Minneapolis, MN, USA) used according to the manufacturer's protocols. Absorbance was recorded at 450 nm using a microplate reader (Bio-Rad Laboratories, Mississauga, ON, Canada) with a wavelength correction set at 550 nm. Sensitivities of the commercial ELISA kits were 0.156 ng/mL, 0.058 ng/mL, and 0.313 ng/mL for MMP-3, MMP-8, and MMP-9, respectively. The concentration of each MMP (active + latent forms) was determined in triplicate assays from two independent experiments.

Determination of MMP-9 activity

Human recombinant MMP-9 was purchased from Calbiochem (San Diego, CA, USA) and prepared at a concentration of 20 μg/mL in 5 mM Tris HCl (pH 7.5), with 10% glycerol, 0.1 mM $CaCl_2$ and 0.005% Brij 35. Activation of MMP-9 was performed in 50 mM Tris-HCl (pH 7.5) with 0.5 mM *p*-aminophenylmercuric acetate for 1 h at 37°C. Activated MMP-9 was diluted to a final concentration of 10 μg/mL in the reaction buffer (50 mM Tris-HCl, 5 mM $CaCl_2$, 200 mM NaCl, and 0.2% Brij 35) and was treated with two-fold serial dilutions of quebecol (500 to 62.5 μM) or without (control). After 15 min of incubation at 25°C, the fluorogenic substrate fluorescein isothiocyanate-labeled DQ™ gelatin (Molecular Probes, Eugene, OR, USA) was added at a final concentration of 50 μg/mL. The assay mixtures were incubated in the dark at 37°C for 2 h before the

fluorescence was measured with a Synergy 2 microplate reader (BioTek Instruments, Winooski, VT, USA), using an excitation wavelength of 490 nm and an emission wavelength of 520 nm. Results were reported as % of activity (means ± standard deviations of triplicate assays from two independent experiments) from a comparison with the control assay without quebecol (set as 100%).

Mineralization activity of osteoblasts

Human osteogenic sarcoma SaOS-2 cells ATCC HTB-85 were purchased from ATCC and routinely cultured at 37°C in an atmosphere of 5% CO_2 in McCoy's 5A medium containing 2 mM L-glutamine and supplemented with 15% heat-inactivated FBS and 100 µg/ml of penicillin G-streptomycin. For the mineralization assay, SaOS-2 cells were cultivated in the above culture medium supplemented with the mineralization activation cocktail (MAC) composed of 10 mM β-glycerophosphate and 0.2 mM ascorbic acid. The cells were seeded at 1 x 10^5 cells/well in a 96-well microplate and incubated for 24 h at 37°C in an atmosphere of 5% CO_2. The medium was replaced with fresh medium containing MAC and two-fold serial dilutions of quebecol (100 to 1.5625 µg/mL). After 5 days of incubation, cells in the wells were fixed with 4% paraformaldehyde and stained with Alizarin Red S (ScienCell Research Laboratories, Carlsbad, CA, USA), according to the manufacturer's protocol. Cells were observed with an inverted Olympus FSX100 microscope (Olympus, Tokyo, Japan). Concentration (in nM) of bound Alizarin Red S was then determined by measuring the absorbance at 405 nm and using a calibration curve. Untreated cells (incubated without quebecol) were used as a control. The mineralization assays were performed in triplicate in two independent experiments.

Statistical analysis

Unless indicated otherwise, all analyses were performed in triplicate in two independent experiments. The data is expressed as means ± standard deviations (SD). Statistical analyses were performed using a one-way or two-way analysis of variance with a *post hoc* Bonferroni multiple comparison test (GraphPad Software Inc., La Jolla, CA, USA). All results were considered statistically significant at $p < 0.001$ or $p < 0.05$.

Supporting Information

Supporting Information: complete tabulated results for the secretion of MMP-3, MMP-8 and MMP-9; proliferation of osteoblast study.

Acknowledgment

This work was funded by the Laboratoire de Contrôle Microbiologique de l'Université Laval (DG; Grant #: 2018-3). The authors gratefully thank Pr Normand Voyer (Université Laval) for his comments on this manuscript and for his contribution in the early design of this study. The authors also wish to acknowledge the contribution of Katy Vaillancourt for her technical assistance and Amy McMackin for proofreading and linguistic review of the manuscript.

Author Information

Corresponding Author

Sébastien Cardinal - *Département de biologie, chimie et géographie, Université du Québec à Rimouski, Rimouski, Québec G5L 3A1, Canada*; orcid.org/0000-0003-4281-2691; Email: sebastien_cardinal@uqar.ca; Telephone : 418-723-1986 ext.1355

Authors

Amel Ben Lagha - *Groupe de recherche en écologie buccale, Faculté de médecine dentaire, Université Laval, Québec, Québec G1V 0A6, Canada*; Present Address : *Département d'immunologie et de biologie cellulaire, Université de Sherbrooke, Sherbrooke J1K 2R1, Canada*; Email: amel.ben-lagha.1@ulaval.ca

Jabrane Azelmat - *Groupe de recherche en écologie buccale, Faculté de médecine dentaire, Université Laval, Québec, Québec G1V 0A6, Canada;* Email: Jabrane.Azelmat@greb.ulaval.ca

Daniel Grenier - *Groupe de recherche en écologie buccale, Faculté de médecine dentaire, Université Laval, Québec, Québec G1V 0A6, Canada*; Present Address : *Département d'immunologie et de biologie cellulaire, Université de Sherbrooke, Sherbrooke J1K 2R1, Canada*; orcid.org/ 0000-0003-0878-4674; Email: Daniel.Grenier@greb.ulaval.ca

References

1. Dye, B. A., Global periodontal disease epidemiology. *Periodontol. 2000* **2012,** *58* (1), 10-25.
2. Curtis, M. A.; Diaz, P. I.; Van Dyke, T. E., The role of the microbiota in periodontal disease. *Periodontol. 2000* **2020,** *83* (1), 14-25.
3. Hajishengallis, G., Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. *Trends Immunol.* **2014,** *35* (1), 3-11.
4. Pan, W.; Wang, Q.; Chen, Q., The cytokine network involved in the host immune response to periodontitis. *Int. J. Oral Sci.* **2019,** *11* (3), 30.
5. Boelen, G.-J.; Boute, L.; d'Hoop, J.; EzEldeen, M.; Lambrichts, I.; Opdenakker, G., Matrix metalloproteinases and inhibitors in dentistry. *Clin. Oral Investig.* **2019,** *23* (7), 2823-2835.
6. Sorsa, T.; Tjäderhane, L.; Konttinen, Y. T.; Lauhio, A.; Salo, T.; Lee, H. M.; Golub, L. M.; Brown, D. L.; Mäntylä, P., Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Ann. Med.* **2006,** *38* (5), 306-21.
7. Nagase, H., Zinc Matrix Metalloproteinases and TIMPs. In *Encyclopedia of Metalloproteins*, Kretsinger, R. H.; Uversky, V. N.; Permyakov, E. A., Eds.; Springer: New York, NY, 2013; pp 2454-2465.
8. Franco, C.; Patricia, H. R.; Timo, S.; Claudia, B.; Marcela, H., Matrix Metalloproteinases as Regulators of Periodontal Inflammation. *Int. J. Mol. Sci.* **2017,** *18* (2).
9. Checchi, V.; Maravic, T.; Bellini, P.; Generali, L.; Consolo, U.; Breschi, L.; Mazzoni, A., The Role of Matrix Metalloproteinases in Periodontal Disease. *Int. J. Environ. Res. Public Health* **2020,** *17*, 4923.
10. Ryan, M. E.; Golub, L. M., Modulation of matrix metalloproteinase activities in periodontitis as a treatment strategy. *Periodontol. 2000* **2000,** *24*, 226-38.
11. Golub, L. M.; Lee, H. M., Periodontal therapeutics: Current host-modulation agents and future directions. *Periodontol. 2000* **2020,** *82* (1), 186-204.
12. Hienz, S. A.; Paliwal, S.; Ivanovski, S., Mechanisms of Bone Resorption in Periodontitis. *J. Immunol. Res.* **2015,** *2015*, 615486..
13. Caetano-Lopes, J.; Canhão, H.; Fonseca, J. E., Osteoblasts and bone formation. *Acta Reumatol. Port.* **2007,** *32* (2), 103-10.
14. Hathaway-Schrader, J. D.; Novince, C. M., Maintaining homeostatic control of periodontal bone tissue. *Periodontol. 2000* **2021,** *86* (1), 157-187.
15. Li, L. Y.; Seeram, N. P., Quebecol, a novel phenolic compound isolated from Canadian maple syrup. *J. Funct. Foods* **2011,** *3* (2), 125-128.
16. Pericherla, K.; Shirazi, A. N.; Rao, V. K.; Tiwari, R. K.; DaSilva, N.; McCaffrey, K. T.; Beni, Y. A.; Gonzalez-Sarrias, A.; Seeram, N. P.; Parang, K.; Kumar, A., Synthesis and antiproliferative activities of quebecol and its analogs. *Bioorg. Med. Chem. Lett.* **2013,** *23* (19), 5329-5331.
17. Gonzalez-Sarrias, A.; Li, L. Y.; Seeram, N. P., Effects of Maple (Acer) Plant Part Extracts on Proliferation, Apoptosis and Cell Cycle Arrest of Human Tumorigenic and Non-tumorigenic Colon Cells. *Phytother. Res.* **2012,** *26* (7), 995-1002.

18. Gonzalez-Sarrias, A.; Li, L. Y.; Seeram, N. P., Anticancer effects of maple syrup phenolics and extracts on proliferation, apoptosis, and cell cycle arrest of human colon cells. *J. Funct. Foods* **2012,** *4* (1), 185-196.

19. Cardinal, S.; Voyer, N., Total synthesis of quebecol. *Tetrahedron Lett.* **2013,** *54* (38), 5178-5180.

20. Cardinal, S.; Azelmat, J.; Grenier, D.; Voyer, N., Anti-inflammatory properties of quebecol and its derivatives. *Bioorg. Med. Chem. Lett.* **2016,** *26* (2), 440-444.

21. Cardinal, S.; Paquet-Côté, P.-A.; Azelmat, J.; Bouchard, C.; Grenier, D.; Voyer, N., Synthesis and anti-inflammatory activity of isoquebecol. *Bioorg. Med. Chem.* **2017,** *25* (7), 2043-2056.

22. Bodet, C.; Chandad, F.; Grenier, D. Inhibition of host extracellular matrix destructive enzyme production and activity by a high-molecular-weight cranberry fraction. *J. Periodontal. Res*. **2007**, *42*(2), 159-68.

23. Durazzo, A.; Lucarini, M.; Souto, E. B.; Cicala, C.; Caiazzo, E.; Izzo, A. A.; Novellino, E.; Santini, A., Polyphenols: A concise overview on the chemistry, occurrence, and human health. *Phytother. Res.* **2019,** *33* (9), 2221-2243.

24. Bunte, K.; Hensel, A.; Beikler, T., Polyphenols in the prevention and treatment of periodontal disease: A systematic review of in vivo, ex vivo and in vitro studies. *Fitoterapia* **2019,** *132*, 30-39.

25. Li, Y.; Jiang, X.; Hao, J.; Zhang, Y.; Huang, R., Tea polyphenols: application in the control of oral microorganism infectious diseases. *Arch. Oral Biol.* **2019,** *102*, 74-82.

26. Fernández-Rojas, B.; Gutiérrez-Venegas, G., Flavonoids exert multiple periodontic benefits including anti-inflammatory, periodontal ligament-supporting, and alveolar bone-preserving effects. *Life Sci.* **2018,** *209*, 435-454.

27. Zappa, U.; Reinking-Zappa, M.; Graf, H.; Espeland, M., Cell populations and episodic periodontal attachment loss in humans. *J. Clin. Periodontol.* **1991,** *18* (7), 508-15.

28. Marcaccini, A. M.; Novaes, A. B., Jr.; Meschiari, C. A.; Souza, S. L.; Palioto, D. B.; Sorgi, C. A.; Faccioli, L. H.; Tanus-Santos, J. E.; Gerlach, R. F., Circulating matrix metalloproteinase-8 (MMP-8) and MMP-9 are increased in chronic periodontal disease and decrease after non-surgical periodontal therapy. *Clin. Chim. Acta* **2009,** *409* (1-2), 117-22.

29. Kinney, J. S.; Morelli, T.; Oh, M.; Braun, T. M.; Ramseier, C. A.; Sugai, J. V.; Giannobile, W. V., Crevicular fluid biomarkers and periodontal disease progression. *J. Clin. Periodontol.* **2014,** *41* (2), 113-120.

30. Meschiari, C. A.; Marcaccini, A. M.; Santos Moura, B. C.; Zuardi, L. R.; Tanus-Santos, J. E.; Gerlach, R. F., Salivary MMPs, TIMPs, and MPO levels in periodontal disease patients and controls. *Clin. Chim. Acta* **2013,** *421*, 140-6.

31. Sexton, W. M.; Lin, Y.; Kryscio, R. J.; Dawson, D. R., 3rd; Ebersole, J. L.; Miller, C. S., Salivary biomarkers of periodontal disease in response to treatment. *J. Clin. Periodontol.* **2011,** *38* (5), 434-41.

32. Chou, Y. C.; Sheu, J. R.; Chung, C. L.; Chen, C. Y.; Lin, F. L.; Hsu, M. J.; Kuo, Y. H.; Hsiao, G., Nuclear-targeted inhibition of NF-kappaB on MMP-9 production by N-2-(4-bromophenyl) ethyl caffeamide in human monocytic cells. *Chem. Biol. Interact.* **2010,** *184* (3), 403-12.

33. Kim Y, Kang H, Jang SW, Ko J. 2011. Celastrol inhibits breast cancer cell invasion via suppression of NF-kB-mediated matrix metalloproteinase-9 expression. *Cell. Physiol. Biochem.* **28**: 175– 184.
34. La, V. D.; Howell, A. B.; Grenier, D., Cranberry proanthocyanidins inhibit MMP production and activity. *J. Dent. Res.* **2009,** *88* (7), 627-32.
35. Huang, H.-T.; Cheng, T.-L.; Lin, S.-Y.; Ho, C.-J.; Chyu, J. Y.; Yang, R.-S.; Chen, C.-H.; Shen, C.-L., Osteoprotective Roles of Green Tea Catechins. *Antioxidants (Basel)* **2020,** *9* (11), 1136.
36. Darveau, R. P.; Hancock, R. E., Procedure for isolation of bacterial lipopolysaccharides from both smooth and rough Pseudomonas aeruginosa and Salmonella typhimurium strains. *J. Bacteriol.* **1983,** *155* (2), 831-8.

Table of Contents Only