EFFECT OF GRAPE COMPONENTS ON PERIODONTAL DISEASE

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Introduction

Half of the adult American population (47 %) are affected by periodontitis, an inflammatory disease which leads to localized destruction of the tissues supporting the tooth. Periodontitis is initiated by periodontal pathogens and caused by excessive host immune responses. *Porphyromonas gingivalis* (*P. gingivalis*) is one of the most important and widely investigated periodontal disease pathogens implicated in the etiology of periodontal disease. Grapes are rich in pro-anthocyanidins which possess extensive biologic properties that have an inhibitory effect on oral bacteria. Besides, pro-anthocyanidins can suppress inflammation. Pro-inflammatory cytokines induced by periodontal pathogens can cause alveolar bone loss and periodontitis. Grapes may be proved as a promising natural therapeutic agent for periodontitis therapy. The focus of this study was to determine the effects of grape components on bacterial growth, inflammation, and osteoclastogenesis *in vitro* and *in-vivo*. Although the mechanism is still unclear, they are believed to target the nuclear factor-kappaB (NF-κB) signal transduction pathway which is an attractive target in many inflammatory diseases like rheumatoid arthritis and cancer. Periodontitis, being a chronic disease of similar inflammatory nature might be benefitted from grape pro-anthocyanidins.

Methods

P. gingivalis was inoculated with grape powder extract and plated on blood agar in anaerobic conditions. Bacterial number was determined by Colony Forming Units (CFU). RAW 264.7 cells were treated with grape powder extract, stimulated with *P. gingivalis* lipopolysaccharide (LPS) and tested for pro-inflammatory cytokine IL-6 by ELISA. Human monocytic THP1-Blue cells™ were treated with grape powder extract and stimulated with LPS. NF-κB-activity in THP1-Blue cells™ was determined by colorimetric activation. RAW 264.7 cells were stimulated with RANKL, differentiated into osteoclasts and treated with grape powder extract. Cultures stained with Tartarate Resistant Acid Phosphatase (TRAP) positive cells were counted. Ligature model mice received a ligation around the second molar and in baker model, *P. gingivalis* was orally infected, and mice consumed a grape powder diet. Gingival tissue was tested for cytokines by qPCR. Bone loss was determined with CEJ-ABC distance.
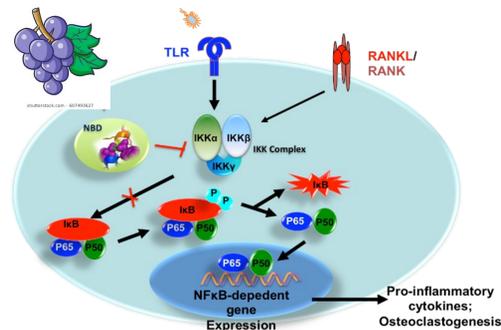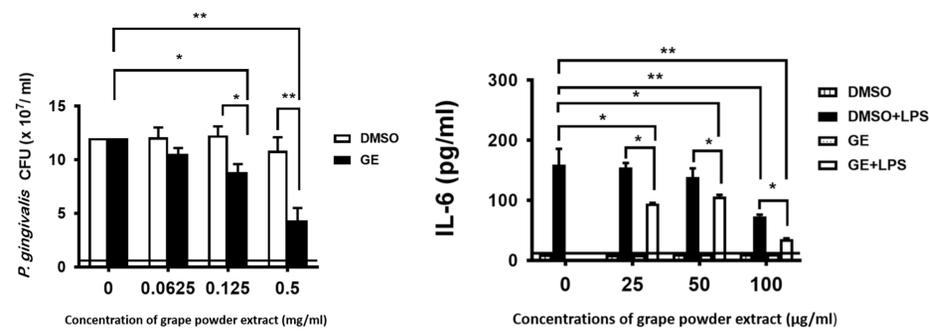

Fig.1 Schematic depiction of the possible working mechanism of grape components The grape components prevent NF-κB nuclear translocation to prevent the NF-κB dependent genes expression. Osteoclastogenesis as well as pathogen-induced inflammation will be inhibited.

Results

Grape powder extract inhibits *P. gingivalis* growth

Figure 2. Graphical comparison of *P. gingivalis* growth after treatment with different concentrations of grape powder extract. *P. gingivalis* were treated with increasing dosages of grape powder extract and incubated anaerobically overnight after which the culture was plated on blood agar plates. After a 7-10-day anaerobic incubation, the plates were counted for colony forming units (CFU). Group of plates inoculated with *P. gingivalis* only and DMSO were the controls. Asterisks indicate statistically significant differences (*, p<0.05; **, p<0.01) between the labelled groups. Data was evaluated by two tailed t test.

Grape powder extract inhibits *P. gingivalis*-induced pro-inflammatory cytokine production

Figure 3. Comparison of different concentrations of grape powder extract and DMSO on *P. gingivalis*-LPS induced pro-inflammatory cytokine IL-6 production in RAW 264.7 cells. RAW 264.7 cells were treated with different dosages of grape powder extract. Cells were incubated at 37 °C for 1 hour and then treated with *P. gingivalis* LPS at a concentration of 1µg/ml. Cells were incubated at 37 °C overnight and supernatant was measured for IL-6 using ELISA. Cells treated with DMSO were used as controls. IL-6 production by the cells without any stimulation nor treatment was labeled out as the baseline. Asterisks indicate statistically significant differences (*, p<0.05; **, p<0.01) between the labelled groups. Data were evaluated by two tailed t test.
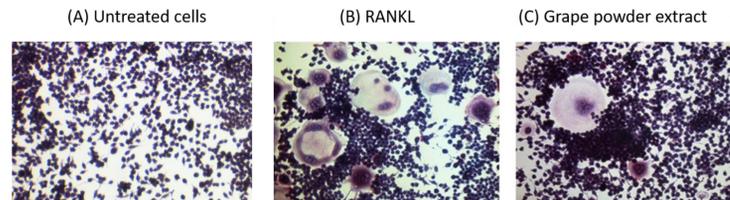

(A) Untreated cells (B) RANKL (C) Grape powder extract

Figure 4. 20x microscopic images showing comparison of number of TRAP positive osteoclasts produced in a 96 well plate in 3 different conditions

Grape powder extract inhibits RANKL-induced osteoclastogenesis A) Untreated RAW 264.7 cells, B) RAW 264.7 cells treated with RANKL only and C) RAW 264.7 cells treated with RANKL and 200 µg/ml of grape powder extract. The number of TRAP positive osteoclasts per well was counted under the electron microscope. Image C depicts a smaller number of osteoclasts in the well (RANKL + grape powder extract) as compared to image B (RANKL only) which reflects higher number of osteoclasts.
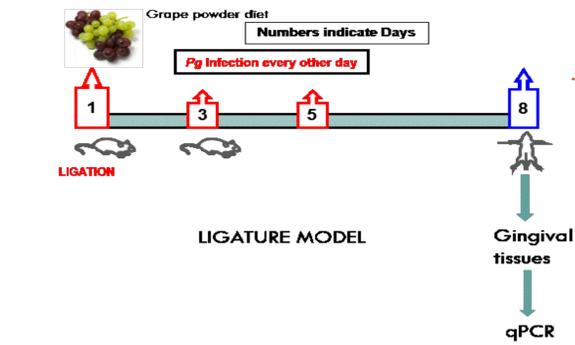
Figure 6. Schematic depiction of the protocol for periodontitis induction in vivo using ligature model on C57BL/6 mice.

The mice were ligated with 6-0 black silk suture on day 1 and infected with 10⁹ colony-forming units (CFU) of *P. gingivalis* (33277) in 2% carboxymethylcellulose (CMC) (day 1, 3 and 5) followed by sacrifice of mice on day 8. The mice were fed with grape powder diet (mouse food fortified with 5% grape powder) or control diet. Gingival tissues were harvested to check expression of pro and anti- inflammatory cytokines by qPCR. Numbers indicate the days.
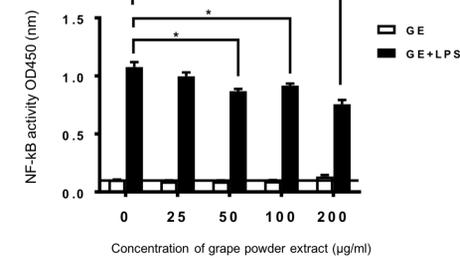
Grape powder extract inhibits *P. gingivalis* LPS-induced NF-kB activation

Figure 5. Comparison of level of *P. gingivalis* LPS-induced NF-κB activation at different concentrations of grape powder extract in THP1-Blue cells™. THP1-Blue cells™ were treated with different dosages of grape powder extract. Cells were incubated at 37 °C for 1 hour and then treated with *P. gingivalis* LPS at a concentration of 10 µg/ml. Cells were incubated overnight, and supernatant was harvested. Cells treated with grape powder extract alone is indicated by the baseline. Activity of NF-κB-inducible alkaline phosphatase was determined by colorimetric activation using a detection reagent Quanti-blue at a 1-hour time point. Asterisks indicate statistically significant differences (*, p<0.05) between the labelled groups. Data were evaluated by two tailed t test.
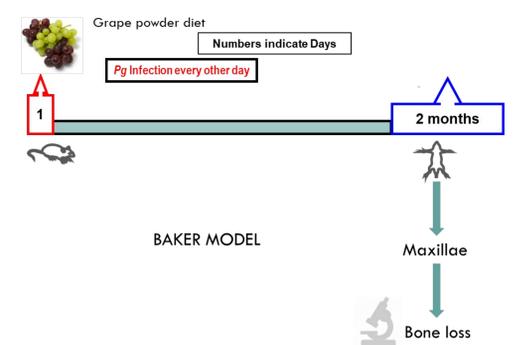
Figure 7. Schematic depiction of the protocol for periodontitis induction in vivo using baker model on BALB/c white mice.

Grape powder diet (mouse food fortified with 5% grape powder) and 10⁹ colony-forming units (CFU) of *P. gingivalis* (3327) oral infection in 2% carboxymethylcellulose (CMC) around maxillary molar teeth 5 times a day for 8 days followed by sacrifice of mice after 2 months and subsequent harvest of maxillary skulls to check bone loss microscopically. Numbers indicate the days.

Fig 9. Microscopic picture of mice maxillary teeth showing the seven sites of measurement from the CEJ to ABC (depicted by black arrows) on the right buccal side after staining with methylene blue for visualization of the CEJ (Baker model).

Fig. 8. Grape powder inhibits alveolar bone loss in a ligature and baker model. Mice were ligated around 2ⁿᵈ molar to induce bone loss. The mice were fed with grape powder diet, no grape powder, or left untreated. The mm distance from the cemento-enamel junction (CEJ) to the alveolar bone crest (ABC) was measured at 6 most affected maxillary buccal sites and the readings were totaled for each mouse. The data are means ± SD (*n* = 5 mice). The CEJ-ABC reading of each mouse was represented by each dot. Bone loss was calculated by subtracting the six-site total CEJ-ABC distance for the ligated mice from the six-site total CEJ-ABC distance of the non-ligated mice. Asterisks indicate statistically significant (*p* < 0.05) differences between grape powder treated and other groups.

Conclusions

Grape components or grape consumption can be beneficial in the prevention or treatment of periodontal disease. Grape components can be useful for further development as a potential and safe therapeutic agent in periodontal disease treatment.
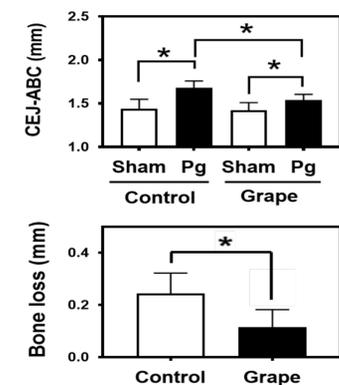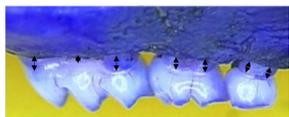
Acknowledgements