Identification of Hop Polyphenolic Components Which Inhibit Prostaglandin E2 Production by Gingival Epithelial Cells Stimulated with Periodontal Pathogen

Hiroaki Inaba, a Motoyuki Tagashira, a, b Daiki Honma, b Tomomasa Kanda, b Yurong Kou, c Yasuuki Ohtake, b and Atsuo Amano a

a Department of Oral Frontier Biology, Osaka University Graduate School of Dentistry; 1–8 Yamadaoka, Suita, Osaka 565–0871, Japan; b Fundamental Research Laboratory, Asahi Breweries Ltd.; 1–1–21 Midori, Moriya, Ibaraki 302–0106, Japan; and c Department of Oral Medicine, School of Stomatology, China Medical University; 117 North Nanjing Street, Shenyang, Liaoning 110002, P. R. China.

Received August 16, 2007; accepted December 6, 2007; published online December 10, 2007.

Chronic marginal periodontitis is a destructive inflammatory disease caused by an imbalance between bacterial virulence and host defense ability, resulting in eventual tooth exfoliation. Porphyromonas gingivalis, a major periodontal pathogen, triggers a series of cellular inflammatory responses including the production of prostaglandin E2 (PGE2), which causes periodontal destruction; thus, anti-inflammatory reagents are considered beneficial for periodontal therapy. In the present study, we examined whether hop- and apple-derived polyphenols (HBP and ACT, respectively) inhibit PGE2 production by human gingival epithelial (HGE) cells stimulated with P. gingivalis components. HGE cells were stimulated with P. gingivalis membrane vesicles, and the effects of HBP, ACT and epigallocatechin gallate (EGCg) on PGE2 production by HGE cells were evaluated using an enzyme-linked immunosorbent assay. HBP and EGCg significantly inhibited PGE2 production, whereas ACT did not. By further fractionation steps of HBP to identify the effective components, 3 components of HBP, 2-[β-(2-methylpropa-noyl)-phloroglucinol]-1-O-β-D-glucopyranoside (MPPG), quercetin 3-0-β-D-glucopyranoside (isoquercitrin), and kaempferol 3-0-β-D-glucopyranoside (astragalin), were found to be elements which significantly inhibited cellular PGE2 production. These results suggest that HBP is a potent inhibitor of cellular PGE2 production induced by P. gingivalis, and HBP may be useful for the prevention and attenuation of periodontitis.

Key words prostaglandin E2; Porphyromonas gingivalis; hop bract polyphenol; humulus lupulus L.; gingival epithelial cell

MATERIALS AND METHODS

Preparation of P. gingivalis Vesicles P. gingivalis ATCC33277 was grown and its membrane vesicles were prepared as described previously. 14 Briefly, bacterial cells were removed from the growth medium by centrifugation at 8000×g for 30 min at 4°C. The culture supernatant was filtered with 0.22 μm pore size, and centrifuged at 100000×g for 1 h at 4°C. The pellet suspended in phosphate-buffered saline (PBS) was used as a vesicle preparation. The protein content of prepared vesicles was determined using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL, U.S.A.).

Cell Culture Immortalized HGE cells 15 were kindly provided by Professor Murakami (Osaka University Graduate School of Dentistry). HGE cells were grown in Humedia KB-2 (Kurabo, Osaka, Japan) at 37°C with 5% CO2, as described previously, 15 and 24 h before stimulation of P. gingivalis vesicles, the cells were moved to Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum.
HGE cells were stimulated with *P. gingivalis* vesicles for 24 h in the presence or absence of polyphenols (HBP, ACT and EGCg) at the indicated concentrations. PGE2 secreted in the culture supernatant was quantified with a competitive ELISA assay. Data show the means ± S.D. (*n* = 9). C, control cells without stimulation of vesicles in the absence of polyphenol; V, HGE cells stimulated with vesicles in the absence of polyphenol. ∗Significant difference (p<0.01) compared with vesicle-stimulated cells (V).

HGE cells were stimulated with *P. gingivalis* vesicles for 24 h in the presence or absence of polyphenols (HBP, ACT and EGCg) at the indicated concentrations. PGE2 secreted in the culture supernatant was quantified with a competitive ELISA assay. Data show the means ± S.D. (*n* = 9). C, control cells without stimulation of vesicles in the absence of polyphenol; V, HGE cells stimulated with vesicles in the absence of polyphenol. ∗Significant difference (p<0.01) compared with vesicle-stimulated cells (V).

HGE cells were stimulated with *P. gingivalis* vesicles for 24 h at the indicated concentrations of LMW-HBP fractions (0%, 5%, 25%, 50% and 80% ethanol (ETOH) fractions; see Materials and Methods for details) PGE2 secreted in the culture supernatant was quantified with a competitive ELISA assay. Data show the means ± S.D. (*n* = 9). C, control cells without stimulation of vesicles in the absence of polyphenol; V, HGE cells stimulated with vesicles in the absence of polyphenol. ∗Significant difference (p<0.01) compared with vesicle-stimulated cells (V).

HGE cells were stimulated with *P. gingivalis* vesicles for 24 h at the indicated concentrations of LMW-HBP fractions (0%, 5%, 25%, 50% and 80% ethanol (ETOH) fractions; see Materials and Methods for details) PGE2 secreted in the culture supernatant was quantified with a competitive ELISA assay. Data show the means ± S.D. (*n* = 9). C, control cells without stimulation of vesicles in the absence of polyphenol; V, HGE cells stimulated with vesicles in the absence of polyphenol. ∗Significant difference (p<0.01) compared with vesicle-stimulated cells (V).
ACT on PGE2 production by HGE cells. The present findings of enzymes involved in PGE2 production, such as phospholipase A2 (PLA2), COX-1 and COX-2, as well as inhibition of the cellular expression of these enzymes, and weakened effect on bacterial virulence factors. MPPG was previously isolated from hops, and shown to inhibit enzymatic activity of PLA2.23) Astragalin is a flavonoid which negligibly inhibited IL-4 expression by blood basophils.24) In addition, proteases secreted by P. gingivalis, abundant in its vesicles, are major factors to induce a host inflammatory response, and HBP effectively inhibited protease activity of P. gingivalis.13) Further investigation is necessary to elucidate the exact pathway to express the PGE2-suppressing activity of HBP.

HBP is reported as a safe food material,25) and HBP and LMWHBP were also shown to be safe for culture cells.13) In contrast, EGCg reportedly showed cytotoxicity at a concentration of more than 50 μM (about 23 μg/ml).26) Our previous study also showed that EGCg caused a significant loss of cellular viability even at a dose of 10 μg/ml.13) In addition to the proven safety, HBP was also capable of protecting periodontal cells from bacterial cytotoxicity,13) as mentioned in the introduction. HBP was previously shown to inhibit cariogenic activity of Streptococcus mutans in vitro,27) and a recent crossover clinical study showed that a mouthrinse containing HBP successfully reduced dental plaque regrowth in humans.28) Caries and periodontitis are two major oral problems; therefore, it is possible that HBP is a candidate not only for a caries-preventing agent, but also for anti-inflammatory reagents with therapeutic potential for chronic periodontitis.

REFERENCES


