Effects of Methanandamide on proliferation and pro-inflammatory mediator or cytokine/chemokine expression of PDLC with or without P. Gingivalis

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Abstract

Endocannabinoids have been indicated to have anti-inflammatory and immune suppressive effects in several tissues, which are involved in a variety of physiological and pathological process both in health and disease. The objective of the present study was therefore to investigate the synthetic cannabinoid methanandamide (Meth-AEA) on proliferation/viability and cytokine/chemokine expression of interleukin-6 (IL-6), interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) in human periodontal ligament cells (HPdLCs) with or without Porphyromonas gingivalis lipopolysaccharide (P. gingivalis LPS) stimulation. Meth-AEA at the highest concentrations of 30μM significantly inhibited proliferation/viability. 10 μM Meth-AEA could decrease IL-6, IL-8 and MCP-1 gene expression and protein level of HPdLCs stimulated by P. gingivalis LPS. The present results demonstrate that Meth-AEA might decrease overproduction of inflammatory cytokine/chemokine in HPdLCs stimulated by P. gingivalis LPS, implying that endocannabinoid might reduce periodontal tissue damage by regulation of excessive inflammatory reaction in periodontitis.

Background and Aim

The endocannabinoid system consists of endogenous lipid ligands, G-protein-coupled cannabinoid receptors, biosynthetic pathways and metabolizing enzymes, which is involved in a variety of physiological and pathological process both in health and disease. However, the exact mechanism of the role of ECS in periodontitis and periodontal healing is little known at present. The accumulating evidence suggests that cannabinoids and their receptors play important roles in the progression of periodontal disease and periodontal healing. Methanandamide (Meth-AEA), a synthetic analogue of anandamide, has a four-fold higher affinity to receptor than anandamide itself and additionally exists a high resistance to enzymatic hydrolysis. Because of the advantages, the synthetic cannabinoids have been created and widely applied for research. In order to elaborate the potentially mechanism of Meth-AEA on the periodontitis treatment, the present study would assessed the Meth-AEA on proliferation/viability and cytokine/chemokine expression of interleukin-6 (IL-6), interleukin-8 (IL-8) and monocyte chemotactic protein-1 76 (MCP-1) in HPdLCs with or without P. gingivalis LPS stimulation.

Methods and Materials

The proliferation/viability of HPdLCs was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method. The gene expression of IL-6, IL-8 and MCP-1 in HPdLCs was measured by real time PCR and the content of corresponding protein in conditioned media was assayed by ELISA.

Results

In this study, we discovered that Meth-AEA at different concentrations (0.03~10μM) exhibited no significantly different effect on the proliferation/viability of HPdLCs, whereas, the highest concentrations of 30μM significantly inhibited proliferation/viability(Fig.1).10μM Meth-AEA could decrease IL-6, IL-8 and MCP-1 gene expression of HPdLCs stimulated by P. gingivalis LPS, whereas, 0.1 and 1μM Meth-AEA showed no influence on gene expression of IL-6, IL-8 and MCP-1 stimulated by P. gingivalis LPS. No significant differences of gene expression of IL-6 and IL-8 can be found without gingivalis LPS induction. However, 10μM Meth-AEA also reduced the MCP-1 gene expression even without P. gingivalis LPS stimulation(Fig.2).10μM Meth-AEA can significantly decrease IL-6, IL-8 and MCP-1 protein level induced by P. gingivalis LPS. Without P. gingivalis LPS stimulation, Meth-AEA did not exhibit any influence on MCP-1 protein production, whereas, IL-6 and IL-8 protein level significantly increased by Meth-AEA of 0.1 and 1μM(Fig.3).

Fig.1. Effect of Meth-AEA on proliferation/viability of HPdLCs. Meth-AEA at different concentrations (0.03~10μM) exhibited no significantly different effect on the proliferation/viability of HPdLCs, whereas, the highest concentrations of 30μM significantly inhibited proliferation/viability.**p<0.01.

Fig.2. Effect of Meth-AEA on gene expression of IL-6, IL-8 and MCP-1 in HPdLCs(A,B,C). Effect of Meth-AEA on gene expression of IL-6, IL-8 and MCP-1 in HPdLCs with P. gingivalis LPS stimulation(D,E,F). *p < 0.05 vs. control group;** p < 0.01 vs. control group;#p < 0.01 vs. Pg LPS;##p < 0.001 vs. Pg LPS

Fig.3. Effect of Meth-AEA on protein production of IL-6, IL-8 and MCP-1 in HPdLCs(A,B,C). Effect of Meth-AEA on protein production of IL-6, IL-8 and MCP-1 in HPdLCs with P. gingivalis LPS stimulation(D,E,F). *p < 0.05 vs. control group;** p < 0.01 vs. control group;#p < 0.01 vs. Pg LPS;##p < 0.001 vs. Pg LPS

Conclusions

The present results demonstrate that Meth-AEA might decrease overproduction of inflammatory cytokine/chemokine in HPdLCs stimulated by P. gingivalis LPS, implying that endocannabinoid might reduce periodontal tissue damage by regulation of excessive inflammatory reaction in periodontitis.

References

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