The Role of Cannabinoid Receptor Type II (CB2R) in Hypertension-induced vascular damage and periodontal disease.

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INTRODUCTION

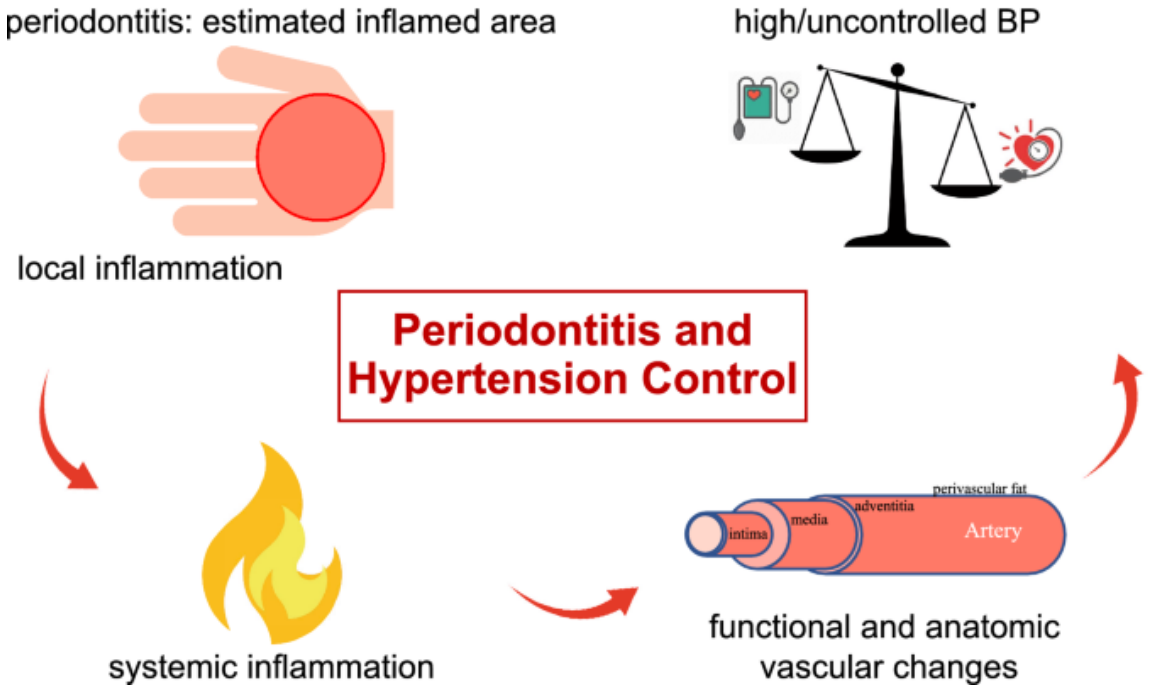
➢Periodontitis (PD) is a major inflammatory disease caused by dysbiotic biofilm affecting soft and hard tissues around the teeth.

➢PD-associated inflammation is not restricted to the oral cavity and is also associated with an increased risk of cardiovascular diseases (CVD)

➢Hypertension is a growing crisis leading to the development and progression of CVD.

➢Many studies have shown periodontitis is associated with hypertension, both are inflammatory diseases characterized by immune cell infiltration and aberrant inflammation.

➢Growing evidence implicates CVD and several other pathologies including oral disease cause dysregulation of both the endocannabinoid system (ECS) and eicosanoids.



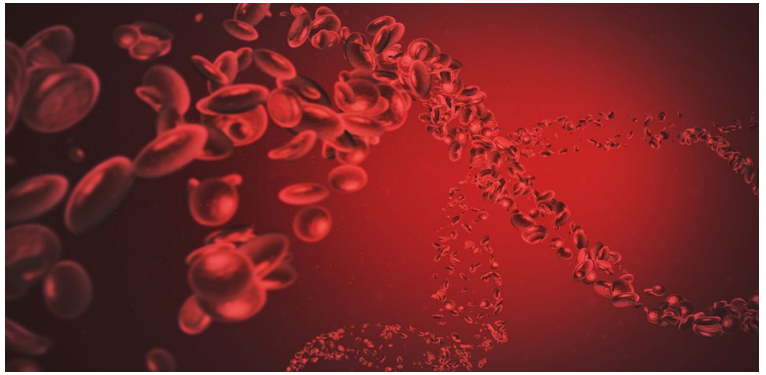
High Blood Press Cardiovasc Prev 27, 281–289 (2020).

BACKGROUND

- Blood vessels play an important role in supplying blood flow to the periodontium and during bone remodeling.

- Excessive inflammation can cause aberrant blood flow due to vascular damage, increased fenestration, and thinning of blood vessels along with nonspecific destruction of the gingiva that leads to eventual bone loss (i.e., periodontitis).

- In chronic inflammation like periodontitis, the vasculature changes that incur may not only increase inflammation but result in vascular dysfunction.



Healthjade.com

BACKGROUND

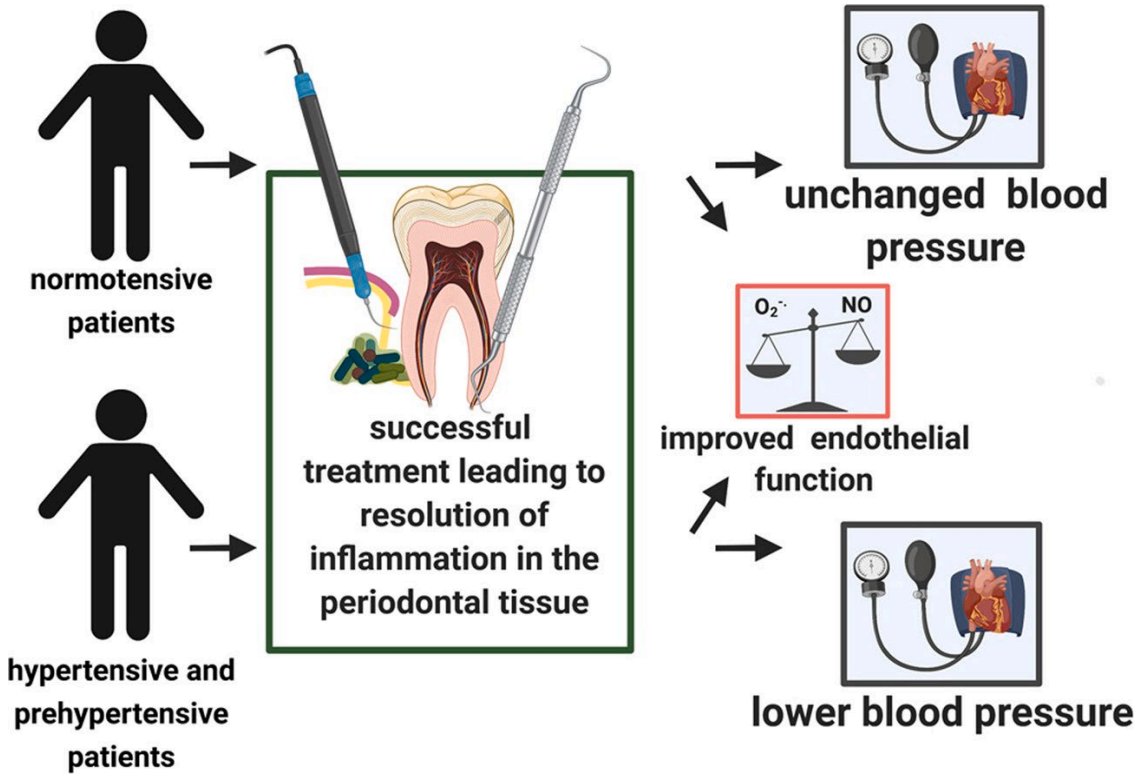
➢Patients with periodontitis often present with higher arterial BP values and have a 30% to 70% higher chance of presenting with hypertension, especially when there is active gingival inflammation (i.e., gingival bleeding).

➢Hypertension is associated with an increased level of inflammation. Thus, hypertension produces excessive inflammation resulting in vascular damage that negatively influences the periodontium.

➢Ang II is a proinflammatory and profibrotic agent that can cause progressive deterioration of disease states and activates intracellular pathways shared with IL-1β, including the NF-κB.

➢It is well known the increase in Ca2+ leads to vascular and blood flow damage. Inositol 1,4,5-trisphosphate receptor 1 (IP3R1) plays an important role in regulating Ca2+ flux from the endoplasmic reticulum (ER) into the cytosol.

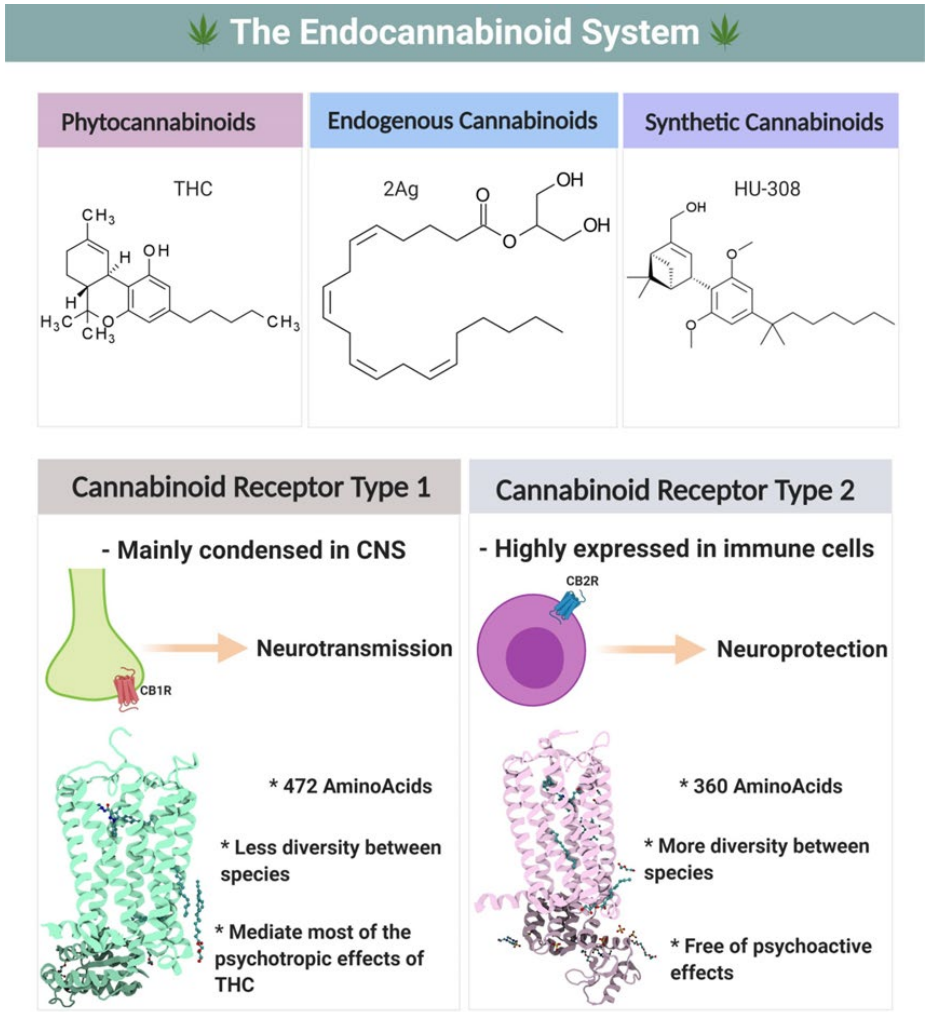
➢Oxidative stress can neutralize nitric oxide (NO) and cause chronic inflammation; in pathophysiological conditions, hyperactivity of NOX induces oxidative stress.



Pharmacol Res. 2021 Apr;166:105511.

THE ENDOCANNABINOID SYSTEM (ECS)

- Cannabinoids Act Mainly via 2 Receptors: Cannabinoid type 1 receptor (CB1R) and Cannabinoid Type 2 Receptor (CB2R)
- CB1R- Expresses predominately expressed on Neurons
- CB2R- Expresses predominately on cells of the immune system & glial neuronal cells



Historically, the medicinal value of *Cannabis* includes the treatment of epilepsy, abscess, tumors, pain, pyretic, vascular function, skin diseases, etc.

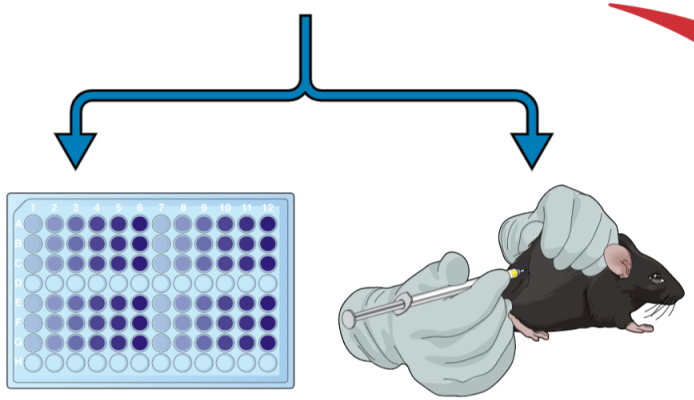
Cannabinoids

Cannabis Sativa

Endogenous cannabinoids

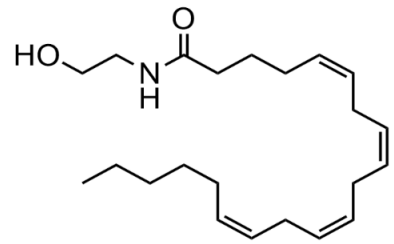
Phytocannabinoids

Synthetic cannabinoids

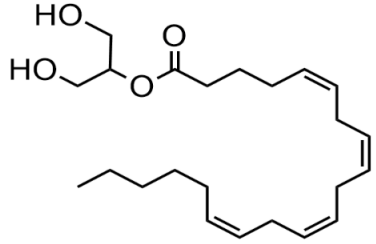


In vitro and In vivo medicinal proprties

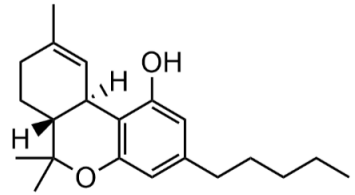
Anandamide



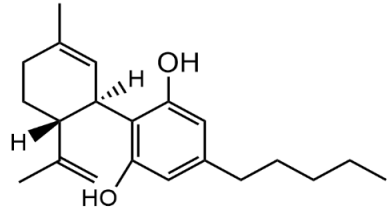
2-Arachidonoylglycerol



D-9-Tetrahydrocannabinol



Cannabidiol



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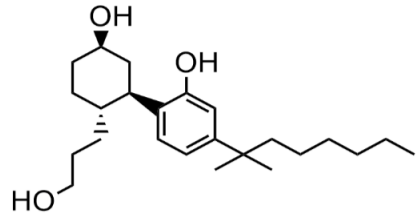

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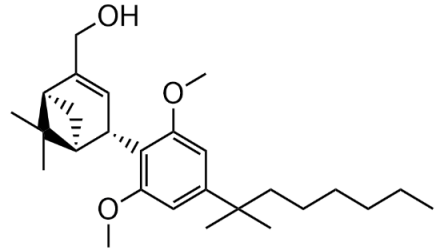
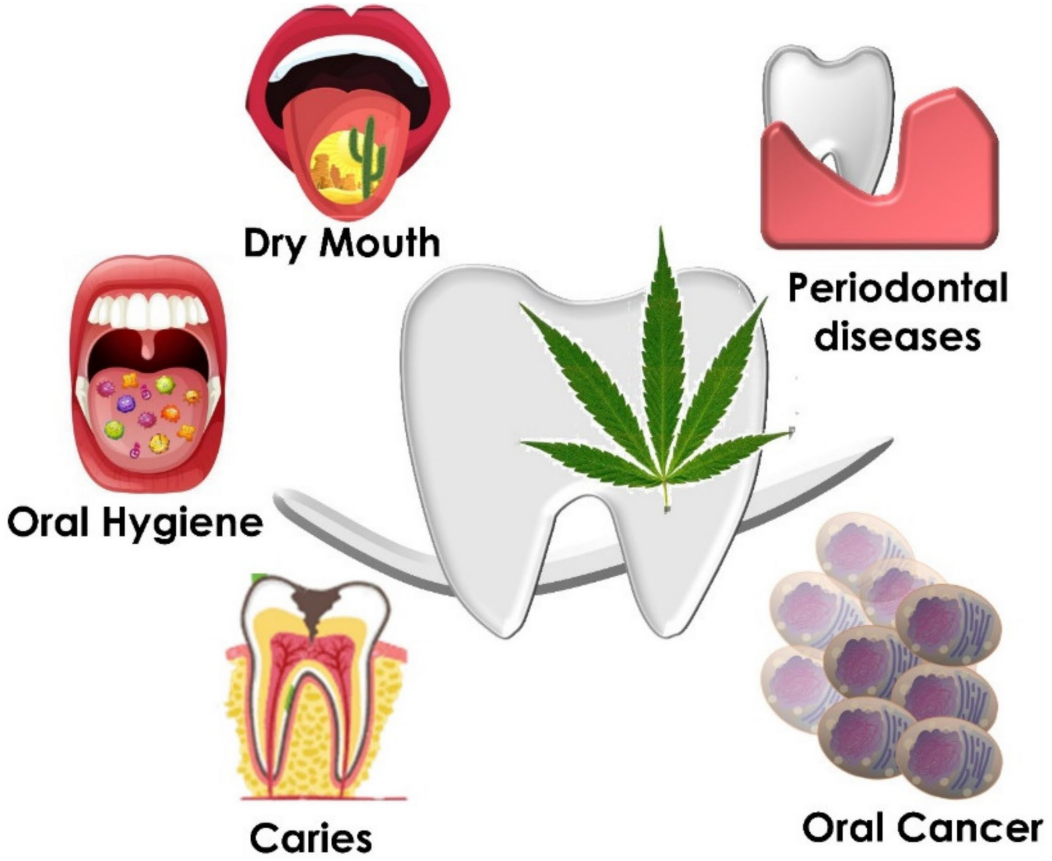


Several studies found the expression of both CB_1 and CB_2 receptors in the human tongue.

Salivary glands express both CB_1 and CB_2 receptors with specific patterns (i.e., CB_1 receptors have been detected in the major salivary glands, while CB_2 receptors instead have been visualized mainly in myoepithelial cells surrounding the acini)

In dental pulp tissues, only, a few reports succeed in the detection of CB_1 receptor expression

Several reports have suggested a role for both CB_1 and CB_2 receptors in pathological conditions, such as inflammation and wound healing



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BACKGROUND

- We previously showed that compared to human clinical studies, primary periodontal ligament (PDL) fibroblasts (hPDLFs) stimulated with IL-1β showed similarities in cytokine and chemokine release.

- In our model, small molecules targeting the endocannabinoid system (ECS), specifically the cannabinoids type 2 receptor (CB2R) resulted in a potent anti-inflammatory effect.

- There is emerging evidence suggesting that the endocannabinoid system plays an important role in cardiovascular regulation and hypertension.

- Recent studies also highlight modulation of the CB2R can relieve proinflammatory cytokines in hypertension.

Check for updates

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ORIGINAL ARTICLE

Journal of PERIODONTAL RESEARCH WILEY

Cannabinoid type-2 receptor agonist, inverse agonist, and anandamide regulation of inflammatory responses in IL-1β stimulated primary human periodontal ligament fibroblasts

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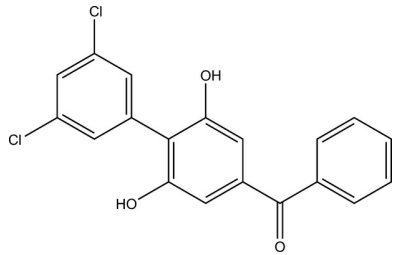
Abstract

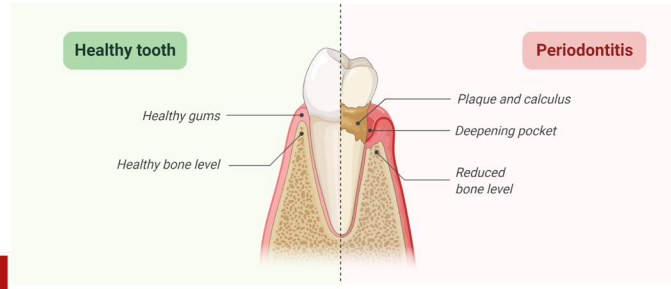
Objective: The aim of this study is to understand the role of cannabinoid type 2 receptor (CB2R) during periodontal inflammation and to identify anti-inflammatory agents for the development of drugs to treat periodontitis (PD).

Background: Cannabinoid type 2 receptor is found in periodontal tissue at sites of



SMM-189

SMM-189 is a potent and selective CB2 inverse agonist



Markers	Roles	PD
INFγ	associated with Huntington's disease and hepatitis C.	YES
IL-1β	involved in aging, wound healing, pyrogenic cytokine, induces prostaglandins, leads to bone destruction.	YES
IL-6	involved in osteoporosis, pulmonary fibrosis, liver cirrhosis, ischemia, and berylliosis among other disorders.	YES
IL-8	associated with acute pancreatitis, ovarian carcinoma, systemic lupus erythematous nephritis H. pylori induced duodenal ulcer and gastritis.	YES
IL-13	involved in allergic rhinitis, inflammatory bowl disease and colorectal cancer.	YES
TNFa	involved in stimulation of other cytokines leading to cachexia, pyrogenic cytokine and associated with inflammation and sepsis while inhibiting tumorgenesis and viral replication.	YES
VEGF/VEGFA	associated with diabetes, renal cell carcinoma, and acute renal allograft rejection along with other disorders.	YES
ICAM	involved in cardiovascular disease, type 2 diabetes, organ transplant dysfunction, and certain malignancies.	YES
VCAM	involved in osteoclastogenesis, induction of sickle cell adherence to vascular endothelial cells during hypoxemia.	YES

Markers	Roles	PD
Eotaxin (CCL11)	associated chronic bronchitis, osteoarthiritis, and colorectal cancer.	YES
Eotaxin-3 (CCL26)	gene is associated with rheumatoid arthritis and allergic asthma.	YES
IP-10 (CXCL10)	involved in chronic obstructive pulmonary disease, multiple sclerosis, and asthma.	YES
MIP-1ᵅ (CCL3)	involved in multiple myeloma, breast cancer, and cystic fibrosis.	YES
MIP-1β (CCL4)	involved granular lymphocyte leukemia, and organ transplant rejection.	YES
MCP-1 (CCL2)	involved in Alzheimer's disease, rheumatoid arthritis and atherosclerosis, among others.	YES
MDC (CCL22)	implicated in atopic dermatitis, and gastric carcinoma.	YES
GM-CSF (CCL11):	involved in autoimmune and inflammatory disorders.	YES
TARC	Atopic dermatitis, systemic lupus erthymatosis, allergic rhinitis, multiple sclerosis, esophageal squamous cell carcinoma.	YES

HYPOTHESIS

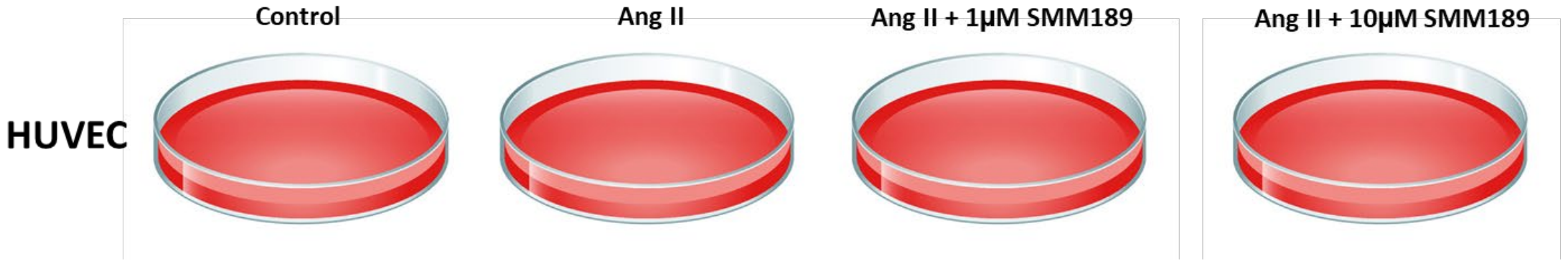
We hypothesize that stimulation of hypertension via angiotensin II will increase inflammation in human umbilical vein endothelial cells (HUVEC). *The endothelial cell damage is negatively associated with blood vessel function*.

In addition, we hypothesized that treatment with a CB2R inverse agonist (SMM-189) will show similar effects as it did in hPDLFs by suppressing inflammation in HUVEC, resulting in blood supply homeostasis to the oral tissues and reduction in PD.

AIMS

Aim 1: Determine if using selective activation of CB2R (SMM-189) will mitigate Ang II-induced inflammation

Aim 2: Investigate if selective activation of CB2R (SMM-189) can modulate inositol 1,4,5-trisphosphate receptors (IP3R) levels.



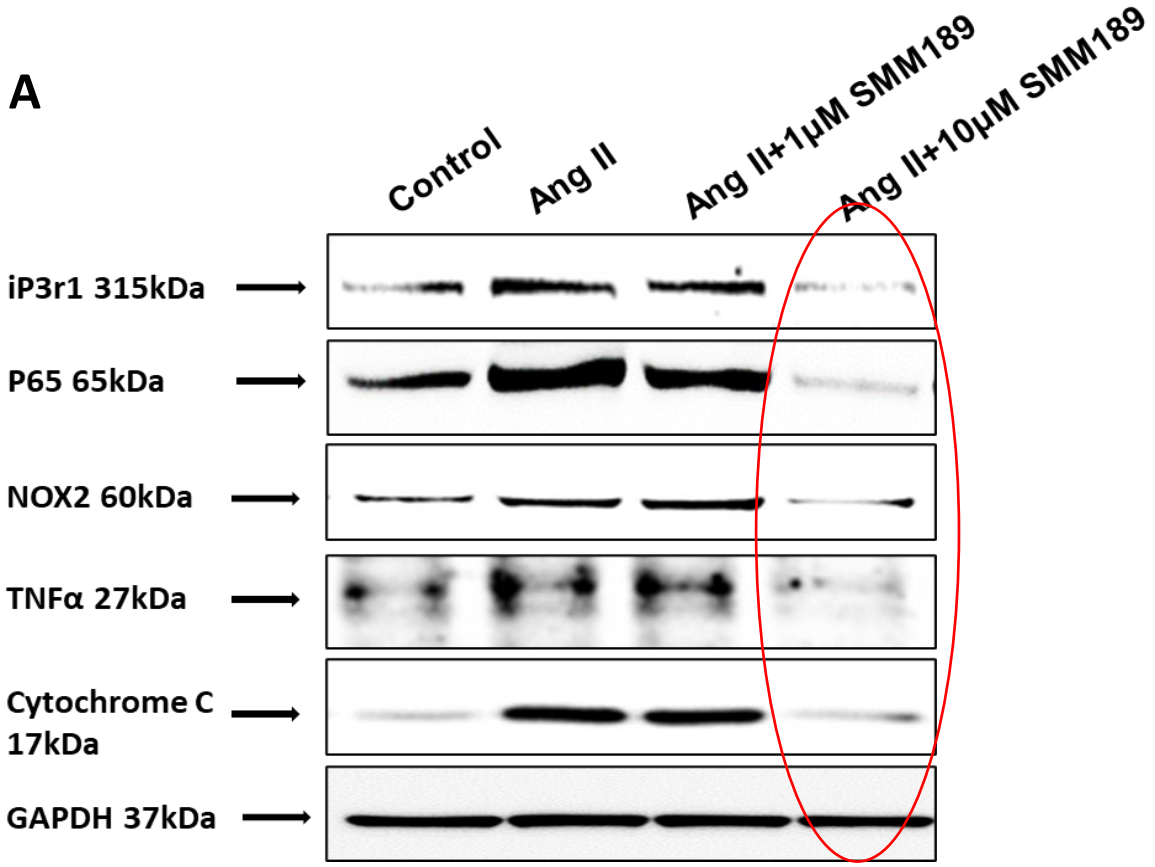
MATERIAL AND METHOD

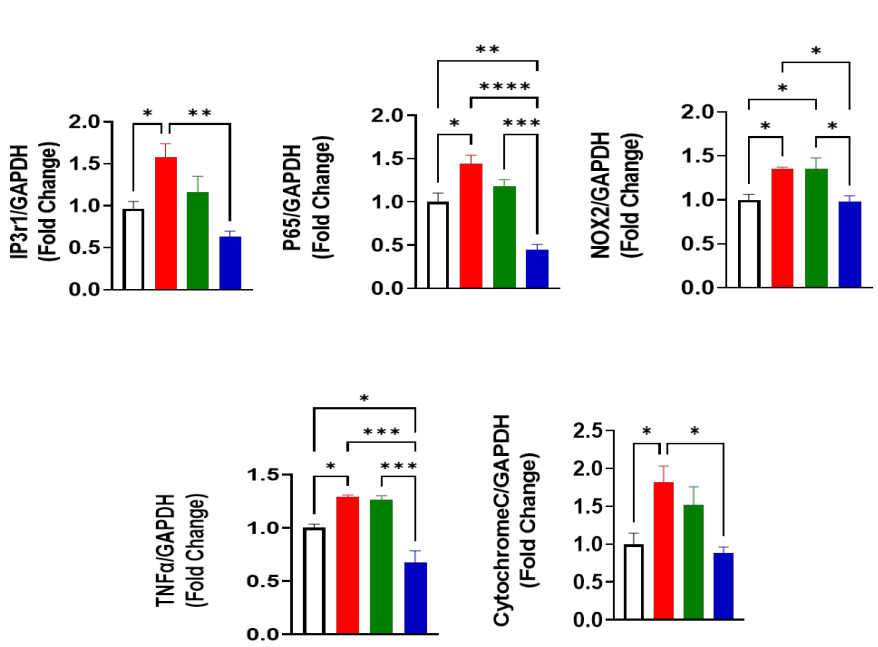
- We used human umbilical endothelial cells (HUEVCS) from American Type Culture Collection (ATCC) grown in Cells Growth Media (ATCC) and treated them with angiotensin II (Ang II) in vitro to mimic hypertension.

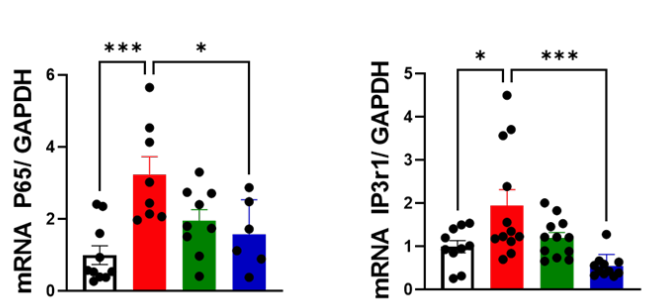
- At 80% of confluency, cells were treated with Ang II (100nM, 24h) in the presence and absence of SMM-189 (1µM and 10µM for 24h).

- After the treatment period, cells were harvested for western blot and RT-PCR.

RESULTS







(A) Western blot, **(B)** western blot quantification, **(C)** mRNA levels for inflammation (p65, TNFα), oxidative stress (cytochrome C, NOX2), and vascular response (IP3r) marker in HUVEC. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

Results Overview

- Ang II significantly elevated IP3R, NFκB (p65), TNFα, cytochrome C, and NOX2 levels in HUVEC.

- Small molecule therapy with SMM-189 (CB2R selective ligand) significantly decreased IP3R, NFκB (p65), TNFα, cytochrome C, and NOX2 in a dose-dependent fashion (1μM and 10μM).

- The importance of IP3R being regulated supports to decrease the elevated maladaptive vascular response that increases vascular tone that is due to the release of Ca2+ in the ER. The ECS by selective modulation can regulate several pathologies this way, including oral disease.

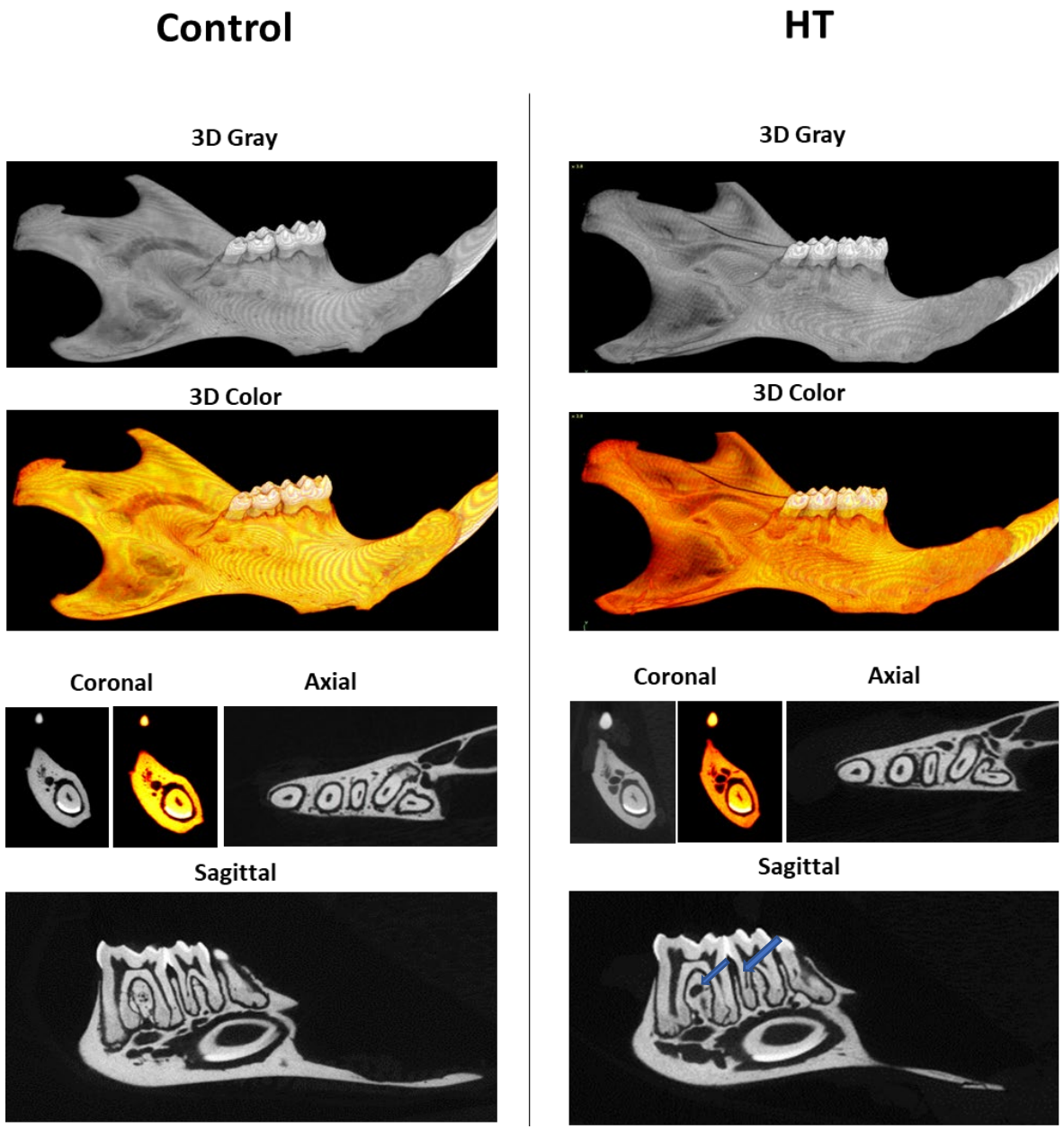
- It may be plausible that activation of ECS by CB2R overcomes the insufficiencies of the endogenous ligands.

Preliminary Animal Study

- Experiments performed on eight-week-old C57/b6 mice.

1. Vehicle group infused with saline.
2. Ang II group infused with Angiotensin II (100 ng/mg/min) using subcutaneous mini osmotic pumps for 4 weeks to mimic hypertension.

- Bodyweight and blood pressure was measured weekly. Liver functions were the determined end of the study. Alveolar bone density measured by µCT 40 (Scanco, Medical AG, Switzerland).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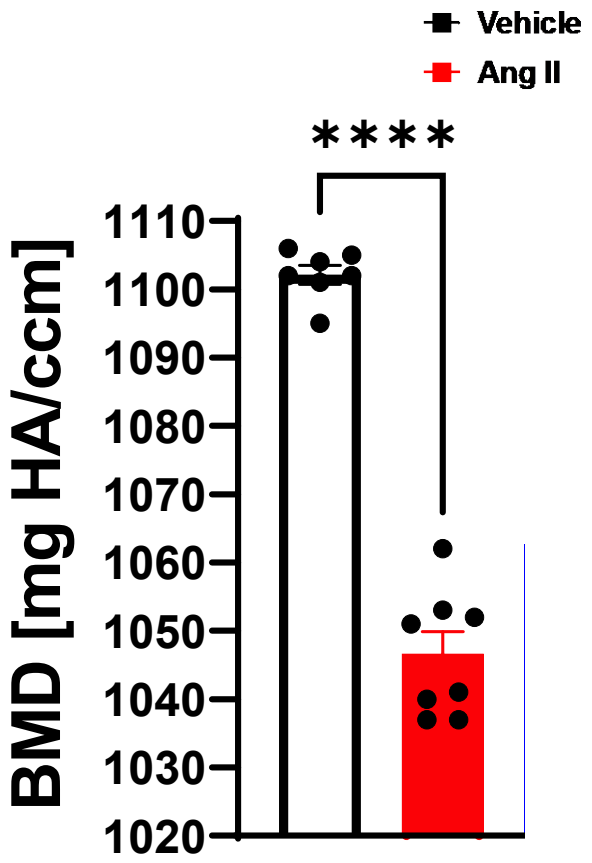
Vehicle
Ang II
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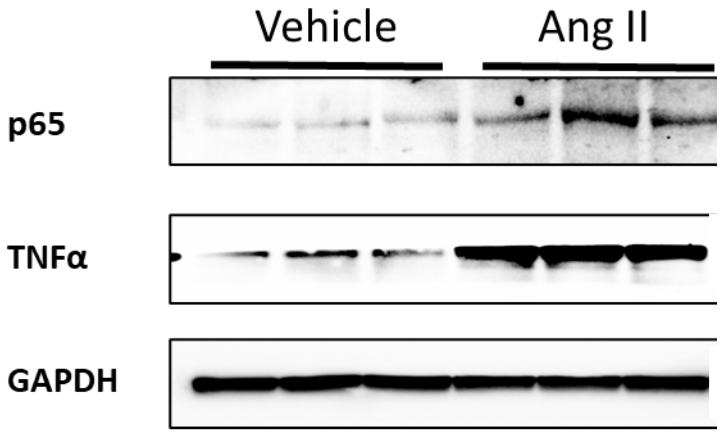
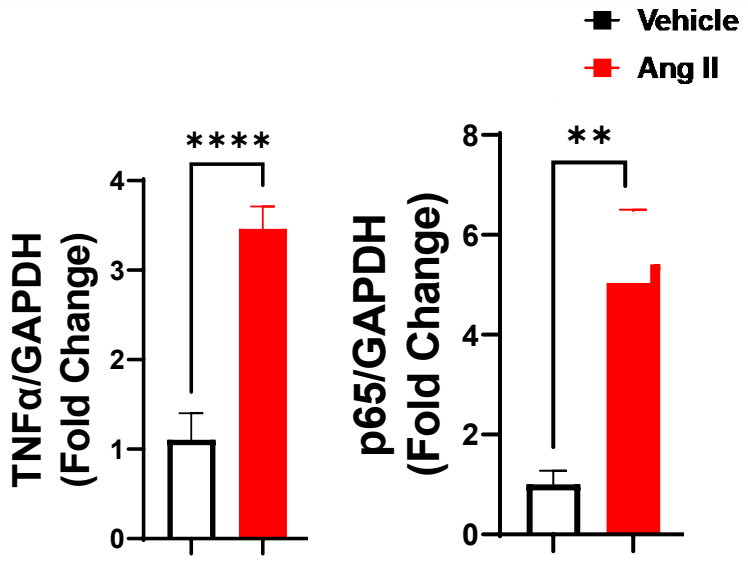
Western Blot : Inflammatory Marker Check





- Western blot and its quantification

FUTURE DIRECTIONS

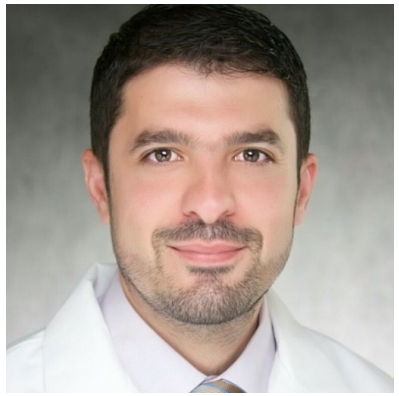
Examining the effect of SMM-189 in Ang-II induced in human vascular smooth (hVSMC)- On going

Initial animal study shows that mice exhibit bone loss on Ang II (induced hypertension).

We will test the effects of SMM-189 on bone loss, blood pressure, vascular contraction, and relaxation.

We will also study Cannabinoid regulation of epigenetics by examining the activity of SMM-189 and its effect on histone deacetylase (HDAC).

ACKNOWLDGMENT



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