Anti-inflammatory activity of cannabinoid receptor 2 ligands in primary hPDL fibroblasts

Article Critique

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Extended Annotated Bibliography (20%)

1. **When was the work published?**

The article “Anti-inflammatory activity of cannabinoid receptor 2 ligands in primary hPDL fibroblasts” by Ammaar H. Abidi, Chaela S. Presley, Mustafa Dabbous, David A. Tipton, Suni M. Mustafa, and Bob M. Moore II was published in the journal “Archives of Oral Biology” in March 2018 (Volume 87).

1. **What are the main points of the article? Write a 150- 200 word summary of the article that accurately conveys the content of the article.**

In the article “Anti-inflammatory activity of cannabinoid receptor 2 ligands in primary hPDL fibroblasts” authors argue that the endocannabinoid system in the human body can be used to reduce the proinflammatory immune response of oral tissues on bacterial load in periodontal patients. They mention the conventional approach in PD treatment and point to its disadvantages such as high cost, consistent patient compliance, and modification of certain habits (smoking). On the other hand the immunomodulation of the tissue response can serve as an alternative method in PD treatment. Abidi et al (2018), in the present study investigate the influence of CB2R agonists HU-308 and AEA, as well as CB2R inverse agonist SMM-189 on inflammatory activity in human periodontal ligament fibroblasts (hPDLFs). The hPDLFs were stimulated with the lipopolysaccharides (LPS) from periodontal pathogen *Porphyromonas gingivalis*, pro-inflammatory cytokines IL-1β, and tumor necrosis factor-alpha (TNF-α), and treated with cannabinoid CB2R ligands. Cytokines IL-6 and MCP-1 were used as inflammation markers in hPDLFs and their concentrations were measured in all groups of cells (control, activated by LPS, TNF-α, and IL-1β, and treated by HU-308, AEA, and SMM-189). The hypothesis that the CB2R inverse agonist (SMM-189) will be equally or even more effective in its anti-inflammatory action comparatively to the CB2R agonists was confirmed by the results of the experiments. The authors concluded that the use of endocannabinoid system can be a promising alternative in development of new medications for the management of inflammation in patients with chronic periodontitis.

1. **Does the work meet the standards to be considered an appropriate/academic/scholarly source? Justify your choice.**

Yes, this article can be considered as an appropriate academic source. “Archives of Oral Biology” is an international peer-reviewed scientific journal established in 1959 and covering research topics in oral biology and craniofacial science. The journal has an impact factor (IF) 1.748 according to the Journal Citation Reports (JCR) database, and 5-Year IF 1.991. The article was received on February 28, 2017, sent back for revision, and finally accepted by international editorial board on December 4, 2017.

1. **Are the qualifications of the author(s) appropriate for an academic article? Briefly describe the authors’ qualifications.**

Yes, the qualifications of the authors are appropriate for an academic article. 5 out of 6 authors hold PhD degrees and work as Assistant or Associate Professors in the University of Tennessee Health Science Center. Their h-indices are in the range from 3 to 18 according to the Scopus database. For instance, the author with the highest h-index (18) Mustafa Dabbous is the Professor of the Department of Microbiology, Immunology, and Biochemistry, College of Medicine, University of Tennessee, Center for the Health Science. He holds a PhD in Biochemistry, and according to his academic CV, he is the author of 18 books in his areas of interest (Cancer Progression and Metastasis, Tumor Cell Motility, Periodontal Disease) (Dabbous M. Kh., Ph.D. CV (n.d). According to the Scopus database he has 51 documents with 1124 citations of his works(In Scopus Database online, 2018).

1. **Is the purpose clearly stated? Restate the purpose of the paper in your own words.**

Yes, the purpose is stated clearly. The authors’ objective was to research the influence of CB2R receptor ligands (AEA, HU-308, and SMM-189) on inflammatory activity of interleukin IL-1β, tumor necrosis factor alpha TNF-α, and endotoxins LPS derived from *P. gingivalis* in human periodontal ligament fibroblasts (hPDLFs) (Abidi et al., 2018). Particularly they proved the hypothesis that the inverse agonist SMM-189 would reduce inflammation more effectively then the agonists HU-308 and AEA.

Experimental Design and Execution (40%)

1. **Is the experimental design clearly described? Describe the design in your own words.**

The experimental design is clearly described in the section “Materials and Methods”. The present study is an in-vitro research performed with the use of real human periodontal ligament fibroblasts. First of all the authors justified their choice of hPDLFs, and then they explained exactly how each group of cells was prepared prior to the tests. The control group contained hPDLFs treated with DMSO (dimethyl sulfoxide), ethanol and dH2O (distilled water). Cytotoxicity for different cytokines was tested under their different concentrations (LPS – 10, 50, 100 and 1000ng/ml, TNF-α- 10 ng/ml, and IL-1β- 1 ng/ml) and was assessed using the CCK-8 assay (Abidi et al., 2018). No cytotoxicity was observed at the maximum doses of all three cytokines. The cells were plated in 96-well polystyrene flat bottom plates with specific growth media (5% FBS – fetal bovine serum, and SCGM Single Quot Kit Suppl. and Growth Factors media). Subsequently the media was changed (antibacterial agents added) to synchronize cell activity, and inflammatory agents added to each plate except the control. Finally the inflammation markers (IL-6 and MCP-1) were measured in every plate: control plates, plates, containing only proinflammatory cytokines, and plates, where hPDLFs were treated with different types of CB2R ligands – every ligand was assessed separately. Therefore in this experimental design independent variables are concentrations of proinflammatory agents as well as the type and the concentration of CB2R receptor ligands, and the dependent variable is the amount of inflammation measured in concentrations of IL-6 and MCP-1.

1. **Have the possible influences on the findings been identified and controls instituted?**

**Describe and evaluate the use of controls and possible influences (spurious variables)**

The authors tried to minimize the influence of unaccounted factors. They emphasized that the hPDLFs were taken from healthy individuals, who were negative for HIV, Hep B, and Hep C. Otherwise the results could be invalid, because these cells could produce unpredictable and compromised immune response. Before some of the plates with cells were treated with anti-inflammatory ligands, antibiotics were added to the media to ensure equal cell activity (in case unaccounted-for bacteria were present in the media. They could produce exotoxins other than those planned for the experimental design). The control plates were used to exclude the influence of unpredicted variables. The p≤0.05 showed that it was done successfully.

1. **Has the sample been appropriately selected (if applicable)? Describe the sample used in the study, and evaluate its appropriateness.**

The selection of fibroblasts for the research was based on the suggestion that these cells with their immune response act as propagators of inflammation in periodontal disease. The exotoxin – lipopolysaccharide (LPS) was derived from the periodontal pathogen *P.gingivalis*, bacteria which are responsible for the progression of periodontal disease. Authors also explained the use of pro-inflammatory cytokines (IL-1β) and inflammatory markers (IL-6 and MCP-1), as according to the previous research they are responsible for bone resorption and destruction of periodontal tissues (Abidi et al., 2018). As the present research is an in-vitro study, the size of the samples wasn’t indicated by the authors

1. **Has the reliability and validity of the article been assessed? Evaluate, and state the test/diagnosis results.**

Yes, the reliability and validity of the results of the study was assessed using one-way ANOVA test. Three parallel biological replicates for each experiment were used. The results are significant at p≤0.05. For particular experiments the results were even more significant with p≤0.01, 0.001, and 0.0001. For example, the results displayed on the Fig. 4b “The effects of SMM-189 on *P.gingivalis* LPS, TNF-α, IL-1β stimulated production of MCP-1 in hPDLFs” indicate that the significant drop in the concentration of MCP-1 is happened due to the introduction of SMM-189 into the media. Significant decreases in expression of MCP-1 were registered in plates: SMM-189+P.g. LPS, SMM-189+TNF-α, SMM-189+IL-1β with p≤0.01, p≤0.001, and p≤0.0001 (Abidi et al., 2018). This means that only SMM-189 could cause the decrease in the concentration of MCP-1 and the chance that another, unaccounted-for factor could do that is less than 1%, 0.1% and 0.001% respectively.

1. **Is the experimental therapy compared appropriately to the control therapy? Describe and evaluate the use of the control group.**

Control samples were compared to the experimental samples during every step of the experiment. As cell activity in every plate was synchronized, the possibly overlooked factors were brought down to a minimum. First, the baseline responses of periodontal ligament fibroblasts when stimulated with LPS, TNF-α, and IL-1β were evaluated. For this purpose the concentration of IL-6 and MCP-1 inflammatory markers were measured. Control samples were left unstimulated and showed very low, almost zero expression levels for IL-6 and MCP-1. Control was also present in every experiment investigating the effect of ligands (AEA, SMM-189 and HU-308) on inflammation level. Plates with control samples of the cells were used to exclude experimental errors and the influence of uncontrolled unaccounted variables, if any.

1. **Is the investigation of sufficient duration? Evaluate, and explain your reasoning.**

As the research was completed in vitro, the duration of the experiments was determined by the time required for the hPDLFs to respond to the stimulation by pro-inflammatory agents and anti-inflammatory ligands. According to the authors, after each experiment the cells were inoculated and incubated with the certain agent and then assayed after 24 hour period. The reason this duration was chosen for the experiment was not mentioned in the article. I assume this is exactly the time required for the typical human periodontal ligament fibroblast to respond to the stimulus either proinflammatory or anti-inflammatory one.

Results and Critique (40%)

1. **Have the research questions or hypothesis been answered? Restate the research questions and/or hypotheses in your own words, and describe if or how they are answered.**

Yes, the hypothesis was clearly answered. Based on the review of the previous research, the authors of the article assumed that the CB2R inverse agonist SMM-189 will be equal or even more effective in its anti-inflammatory action in hPDLFs. In order to prove this assumption they used three types of proinflammatory cytokines (LPS, TNF-α, and IL-1β) on hPDLFs. After that they evaluated cells’ response by registering the levels of inflammatory markers (IL-6 and MCP-1) and then treated them with agonist CB2R ligands (HU-308 and AEA), and inverse agonist ligands (SMM-189). The levels of IL-6 and MCP-1 were measured again. The results of the study were presented in the form of graphs. According to the results the cells threated with SMM-189 showed the most significant decrease in IL-6 and MCP-1 expression meaning that this type of CB2R ligand suppresses inflammatory pathways in hPDLFs more effectively. This conclusion confirmed the initial hypothesis.

1. **Do the interpretations and conclusion logically follow the experimental finding?**

**Restate the conclusion, and explain if or how they follow the experimental findings.**

Yes, the interpretations and conclusion logically follow the experimental findings. And the overall composition of the article is very well planned and organized. The conclusion is very clear, concise, and straight to the point. It paraphrases the hypothesis stated in the beginning. The main three points are outlined in the conclusion. First of all, the authors (Abidi et al., 2018) emphasize that their study proved their assumption about effective use of CB2R ligands as anti-inflammatory regulators in periodontal disease. Secondly, the positive effect was shown by both agonists and inverse agonists in the present study, but inverse agonist showed more effective results. And finally the authors concluded that the perspective research of the ligands for endocannabinoid receptors (CB2R) may lead to elaboration of a new effective drug for the treatment of chronic periodontitis. These points completely follow the experimental findings stated in the result section.

1. **Do you agree or disagree with the article and findings? Explain why?**

I agree with the article and its findings, as the experiment was very thoroughly designed. Each section of the article was very well structured and had a logical connection with the previous one. The hypothesis and concluding ideas were stated clearly, the results were represented very demonstrably and explained in detail in the discussion section. The authors reviewed a large amount of resources prior to their study and pointed the areas which needed additional research and could have beneficial findings, especially for the treatment of periodontal disease. The validity and reliability of the findings was statistically confirmed (p≤0.05, and even more accurate for some experiments). Therefore, I believe that this article meets all requirements for the research report, and presents real scientific value.

1. **What would you change in the article? Why? Think outside of the box. What would you add or delete.**

Cannabinoid receptors (CB2R and CB1R) can be activated by three types of ligands: endocannabinoids (produced in human body), plant cannabinoids (cannabidiol), and synthetic cannabinoids (produced in lab). In this article the authors investigated the anti-inflammatory action of two of them. AEA – is produced in human body, and HU-308 and SMM-189 were both prepared in labs. The only type of cannabinoids the authors didn’t consider for this research – plant cannabinoids, particularly cannabidiol. I think it would be very interesting to find out the effectiveness of this type of ligands, and compare them to other types. I would also add them to the experimental design. The authors didn’t explain the reason why natural plant cannabinoid weren’t used and didn’t discuss their potential use in further research.

References

Abidi, A.H., Presley, C.S., Dabbous, M., Tipton, D.A., Mustafa, S.M., & Moore, B.M. (2018). Anti-inflammatory activity of cannabinoid receptor 2 ligands in primary hPDL fibroblasts. *Archives of Oral Biology, 87*, 79-85. <https://doi.org/10.1016/j.archoralbio.2017.12.005>

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