Effect of Photobiomodulation in Sonic Hedgehog pathway inhibited rat submandibular gland

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Abstract

- **Objective:** The submandibular gland (SMG) produces the most saliva, and factors like aging and chemotherapy can affect its structure and function. However, there are only temporary treatments available for salivary hypofunction. This study aimed to evaluate the effects of photobiomodulation (PBM) to function of SMG by using a rat animal model and Vismodegib, a antagonist of sonic hedgehog(SHH) pathway
- **Methods:** Vismodegib drug was gavaged orally for 14 days to significantly decrease the sonic hedgehog signaling proteins (SHH, PTCH1, SMO, GLI1), induce structural changes, and affect salivary functional markers AQP5 and KRT5 in SMG. After that, in conjunction with Vismodegib administration, PBM was performed using an 850 nm high-power LED device treated daily for six days at varying total energy densities of 60, 120, and 180 J/cm^2
- **Results:** Significant increases in the expression of SHH-related proteins with the recovery of SMG ductal cells damaged after Vismodegib administration were observed for PBM-treated groups. Salivary functional marker AQP5, KRT5 also increased in all PBM-treated groups
- **Conclusions:** This study found that Vismodegib decrease SHH-related proteins and associated salivary functional markers, also 850 nm high-power LED can increase the expression of these proteins and markers. Also, PBM recovered the damaged structure of SMG induced by Vismodegib. The study results suggest that PBM can restore SMG structure and function through SHH signaling

Introduction

- Submandibular gland(SMG) produces the most saliva in humans, which is essential for digestion, oral hygiene, preventing infection, and maintaining cell quality
- Chemoradiotherapy is a primary treatment for head and neck cancer (HNC), and SMG is sensitive to radiation due to the slow proliferation of its epithelial cells
- The number of HNC patients are increasing but there are only temporary solutions such as artificial saliva or saliva-secretion stimulator for salivary hypofunction
- Studies have reported that Photobiomodulation(PBM) irradiation of salivary glands was effective in patients with xerostomia or impaired salivary gland function
- Sonic hedgehog(SHH) signaling pathway plays a role in salivary branching and morphogenesis during embryonic development and also regeneration of adult salivary gland
- This study aims to evaluate the effect of Vismodegib and PBM in rat submandibular gland by regulate SHH signaling

Methods and Materials

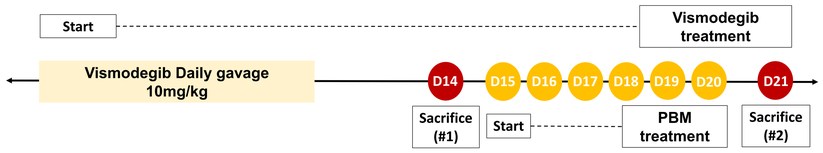
- 20 SD rats (120-140g, 5-week-old male) for each experiments
- Conducted according to the Institutional Animal Care and Use Committee guidelines at Dankook University

Vismodegib

- Control / Vismodegib group
- Vismodegib 10mg/kg daily
  #1 - 14 days and sacrifice
  #2 - 21 days(6 days with PBM) and sacrifice

Photobiomodulation

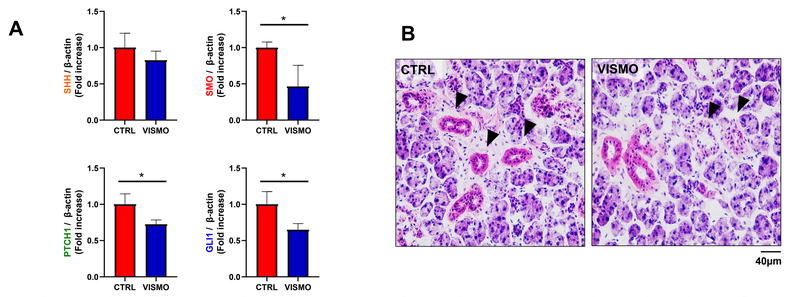
- 850nm high-power LED
- 6 days(with Vismodegib)
- Power density : 65.2 mW/cm^2
- Sham / 60J / 120J / 180J group
  - 60J /cm^2 : 154 sec daily(10J/cm^2 per day)
  - 120J /cm^2 : 307 sec daily(20J/cm^2 per day)
  - 180J /cm^2 : 461 sec daily(30J/cm^2 per day)



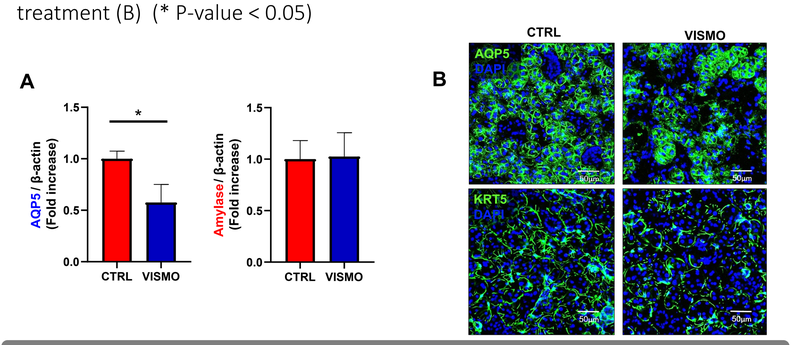
Results

Effect of Vismodegib

- Vismodegib (VISMO) treatment significantly decreased SHH-related protein expressions (SMO, PTCH1 & GLI1) based on the western blot analysis (A) and disrupted ductal cells (B, arrow heads) in rat submandibular glands as shown in the H&E-stained tissue sections (* P-value < 0.05)



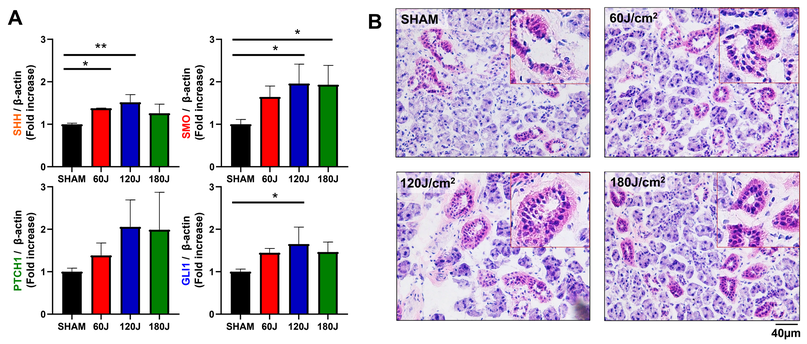
- Salivary gland functional markers (AQP5 & amylase) were evaluated and significant decrease in AQP5 protein expression was observed after VISMO treatment in SMG (A) and confirmed with corresponding immunofluorescence staining (B)
- Keratin5 (KRT5) expression was also found to have decreased after VISMO treatment (B) (* P-value < 0.05)



Effect of Photobiomodulation

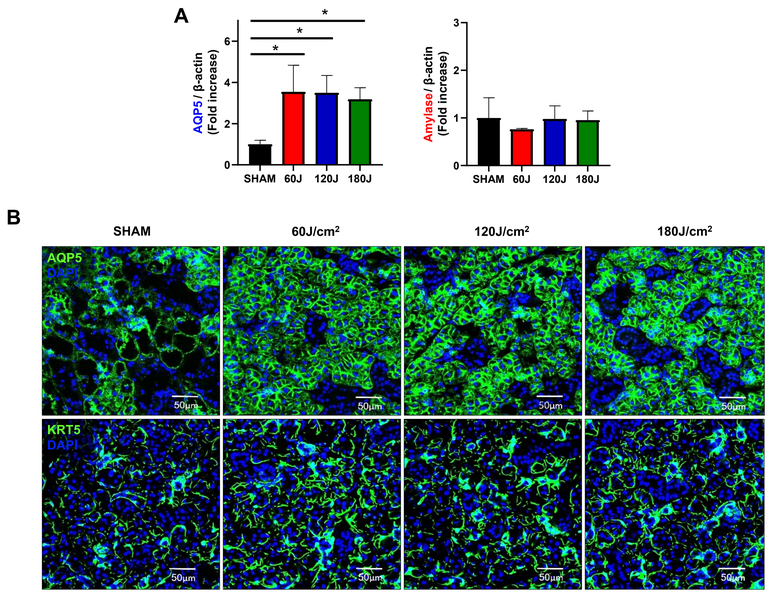
- Relative protein expressions of SHH, SMO and GLI1 significantly increased in SMG tissue after PBM treatment in western blot analysis (A)
- H&E-stained images of extracted tissues show increased cell proliferation and regeneration of the cytoplasm of SMG ductal cells after PBM treatment.(B)

(* P-value < 0.05, ** P-value < 0.01)



- PBM treatment resulted in the recovery of AQP5 protein expression a salivary functional marker as shown in the western blot data (A) and confirmed with the immunofluorescence images (B).
- KRT5 fluorescence expression was also found to be higher for PBM treatment groups compared with SHAM group (B)

(* P-value < 0.05)



Discussion

- Limitations of the study include the small sample size and this study has not identified a specific mechanism of how PBM activates SHH signaling, also while human salivary gland cells are divided into serous and mucous cells, rat salivary gland cells are mucoserous cells
- Further studies are needed with a larger size, variable wavelength, and mRNA sequencing are needed to delineate the precise mechanism involved in SHH-mediated SMG regeneration using PBM therapy
- This study has novelty: There has been a limited report on the effects of Vismodegib, particularly on the submandibular gland, and the relevance of SHH signaling. No previous study shows that PBM can regenerate SMG through activating SHH signaling

Conclusions

- The results showed that Vismodegib significantly decreased the expression of SHH signaling-related proteins and caused structural damage, particularly to the ductal cells in the SMG
- PBM treatment (850nm high-power LED) recovered the expression of the same proteins and ductal gland structure
- These findings suggest that PBM can contribute to new solutions for salivary dysfunction and provide a practical, safe, noninvasive therapeutic approach

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Reference

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