Investigating the Therapeutic Potential of Piperlongumine Derivatives for Noise-induced Hearing Loss

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ABSTRACT: Preliminary evidence from our lab suggests that **piperlongumine** – a natural product from the Indian long pepper (*Piper longum*) – **has therapeutic potential against noise-induced hearing loss (NIHL)[1,2].** When hair cells in the inner ear are exposed to excessive noise, there's an increase in neurotransmitters released, **which results in inflammation (activation of the NF-κB pathway) and cell death[3,4].** It has been shown **that piperlongumine regulates the NF-κB pathway in cancer systems[1].** Similarly, piperlongumine may be protecting the noise-injured hair cells by inhibiting NF-κB**. Thus, we propose that by modifying the chemical structure of piperlongumine, we will 1) improve its efficacy for protection against NIHL; and 2) increase its potency at inhibiting the NF-κB pathway.**
We synthesized and screened 34 piperlongumine derivatives in a zebrafish model for NIHL. We measured the degree of NF-κB inhibition by piperlongumine and its derivatives in an NF-κB zebrafish reporter line and in mouse embryonic fibroblasts. One-way ANOVA was used as our statistical analysis.
We found five derivatives that significantly protect cells against NIHL. These derivatives performed better than the original piperlongumine molecule. Moreover, piperlongumine and its protective derivatives prevented cell death by, at least in part, inhibiting the NF-κB pathway.

Background

- 466 million people globally have disabling hearing loss, and that number is expected to rise to over 700 million by 2050.[5]
- The degradation of the inner ear from acquired or age-related hearing loss produces a total cost of $980 billion annually.[5]
- **We propose that by modifying the chemical structure of piperlongumine (Figure 1), we will 1) improve its efficacy for protection against NIHL; and 2) increase its potency at interfering with the NF-κB pathway (Figure 2).**

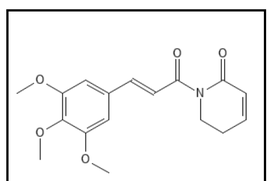

Figure 1. We synthesized 34 derivatives from this original piperlongumine molecule.

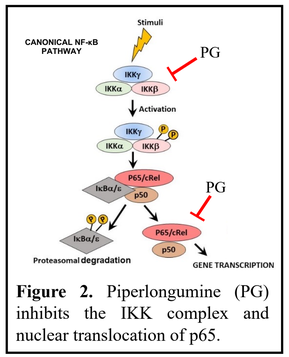

Figure 2. Piperlongumine (PG) inhibits the IKK complex and nuclear translocation of p65.

Materials and Methods

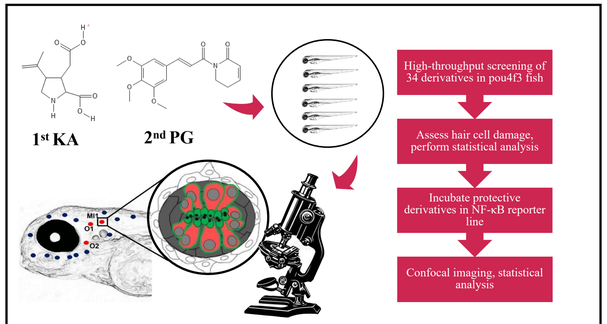

Figure 3. The experimental process we used to find protective derivatives and assess their interactions with the NF-κB pathway. Modified from Ingersoll et al., 2020.

- **Western blot:** Mouse embryonic fibroblasts (MEFs) were incubated with piperlongumine, PG18, PG53, or PG54 (75μM to 1nM) for 1 hour followed by a 30 min incubation with TNF-a. Membranes were immunoblotted for phospho-p65 (ph-p65) and re-probed for total p65 (t65). The results are presented as the ratio between ph-p65/tp65.

Results

High-throughput drug screens of the 34 derivatives found 5 significantly protective derivatives.

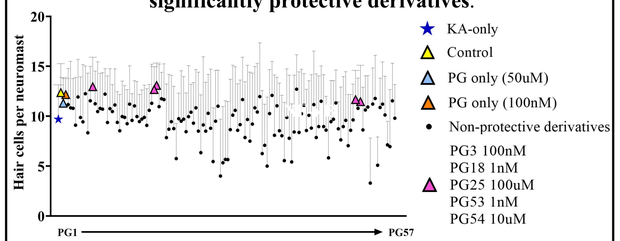

Figure 4. Drug screen results are expressed as Mean + SD.

The structures of the protective derivatives may offer insight into their efficacy.

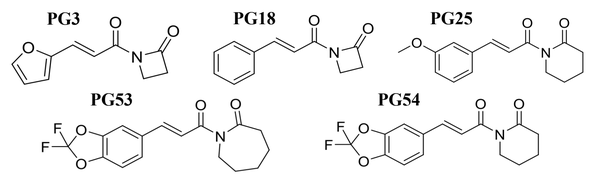

Figure 5. PG3 and PG18 contain an azetidine group. PG53 and PG54 contain fluorine. PG25 is the most similar to PG; the leftmost heterocycle has fewer side groups, so it may have favorable steric and electronic effects.

In MEFs, PG54 inhibited the NF-κB pathway at a lower concentration than piperlongumine.

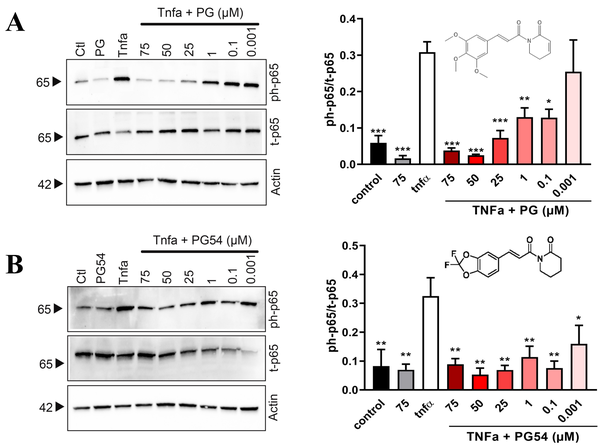

Figure 8. Representative immunoblots of MEFs treated with PG (A) or PG54 (B) at various concentrations for 1 hour followed by co-treatment with TNFa for 30 minutes. The activation of the NF-kB canonical pathway was evaluated by the levels of p65 phosphorylation (ph-p65). Results are presented as the ratio between ph-p65:total-p65 (t-p65).

The five protective derivatives are more potent than the original piperlongumine molecule.

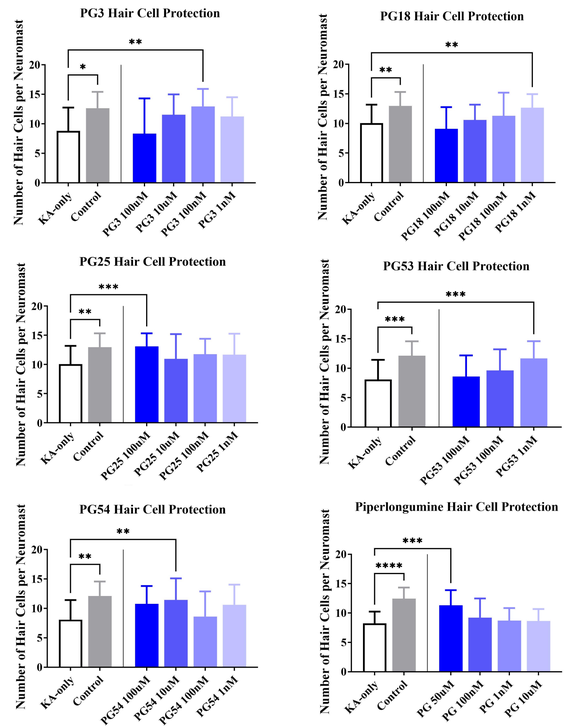

Figure 6. Fish were pre-incubated with KA for 60min, followed by a 2hr incubation with the corresponding derivative. Results are expressed as Mean +/- SD. Statistical analysis: One-way ANOVA followed by Dunnett's post-test. The mean of each treatment was compared with the mean of the KA-only group, which served as the baseline for maximum damage from excitotoxicity.

PG25 and PG54 significantly inhibited the NF-κB pathway in neuromasts.

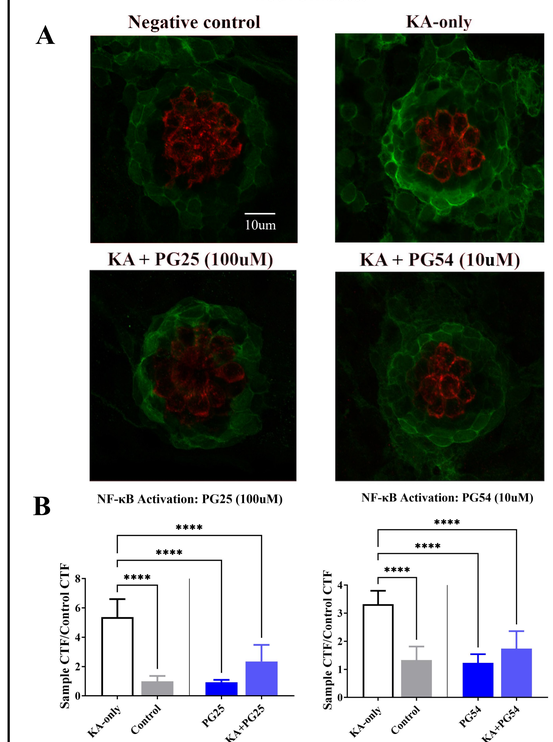

Figure 7. A) Neuromast images of zebrafish and their respective treatments. The negative control establishes the basal level of NF-κB activation in a healthy neuromast, while fish treated with KA-only establishes the highest levels of NF-κB activation. **B)** Quantification of neuromast hair cells after treatment. Results are expressed as Mean +/- SD. Statistical analysis: One-way ANOVA followed by Dunnett's post-test.

Conclusions

- 5 piperlongumine derivatives significantly protect neuromast hair cells against kainic acid-induced excitotoxicity
- Piperlongumine and its otoprotective derivatives prevent hair cells from undergoing apoptosis by, at least in part, inhibiting the NF-κB pathway
- Piperlongumine, PG25, and PG54 all showed significant inactivation of the NF-κB pathway. In MEFs, PG54 had significant inactivation at the lowest concentration tested

Future Directions

- Establish the EC50 and the minimum lethal dose for the top derivatives to calculate their therapeutic windows
- *In vivo* testing of the top derivatives in a mouse model for NIHL

References

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Acknowledgements

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