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Epalestat Prevents the Defects of Sensory Nerves of Lateral Line in Zebrafish Peripheral Nervous System

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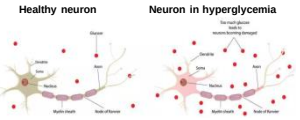
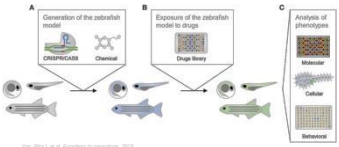
INTRODUCTION

Diabetic peripheral neuropathy(DPN)



- **Diabetes mellitus(DM)**
  : A metabolic disorder characterized by high levels of blood sugar(hyperglycemia) due to very little production of insulin or insulin resistance.
- **Diabetic peripheral neuropathy(DPN)**
  : One of the most common complications of DM.
  : Degeneration of sensory and motor axons by chronic hyperglycemia.
- However, DPN has been poorly understood in the clinical setting, and there is little treatment for DPN

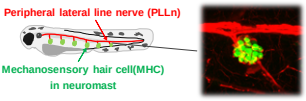
Zebrafish is an ideal tool for the drug screening



Xiao, Ritao Li, et al. Frontiers in neurology, 2018

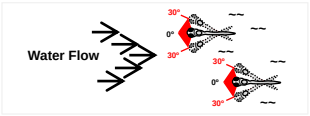
- **Advantages**
  : Vertebrate animal model having genetic similarity to mammal
  : Easily accessible for genetic engineering (Transgenesis, CRISPR/Cas9 mutagenesis)
  : Rapid embryo development
  : Transparency of zebrafish embryo that allows live-cell imaging
  -> High throughput chemical screening

Peripheral lateral line system in zebrafish



- The peripheral lateral line(PLL) is a sensory system used to detect changes of water flow
- The PLL is composed of PLLn and MHC

➢Zebrafish Rheotaxis behavior



- Rheotaxis is an innate sensory behavior that orient toward a water flow and swim to hold position within 30-degree angle
- Peripheral lateral line system is involved in the rheotaxis behavior

Figure 1.

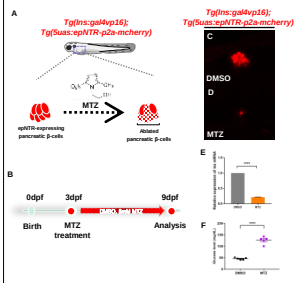


Figure 1. Chemogenetic ablation induces hyper- glycemia in *Tg(ins:gal4vp16);Tg(5uas: epNTR-p2a-mCherry)* zebrafish. (A) Schematic representation about ablation of pancreatic β-cells using epNTR/MTZ system. (B) Schematic timetable of MTZ treatment for hyperglycemia induction. (C,D) Fluorescence images of pancreatic β-cells in DMSO (C) and MTZ-treated transgenic zebrafish (D) at 9days post-fertilization (dpf). White arrow indicates the mCherry+ insulin in pancreatic β-cells (C). (E) Relative expression of *ins* mRNA from DMSO and MTZ-treated transgenic zebrafish. ****p< 0. 0001; DMSO vs. MTZ (n=15 larvae per group). (F) Glucose levels in DMSO or MTZ-treated transgenic zebrafish. ****p< 0.0001; DMSO vs. MTZ (n=100 larvae per group).

Figure 2.

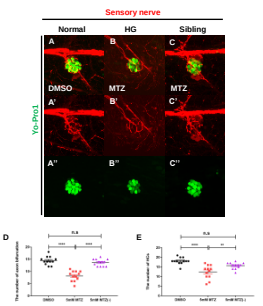


Figure 2. Hyperglycemia causes loss of peripheral lateral line nerve and mechanose-nsory hair cells in hyperglycemic zebrafish. (A-C) Fluorescence images of sensory nerves and mechanosensory hair cells(MHCs) in PLL of normal (A,C) and hyperglycemic(HG) zebrafish (B) at 9days post-fertilization (dpf). (D) Statistical analysis of the number of axon bifurcation from panel A-C. ****p< 0.0001; DMSO vs. MTZ, MTZ vs. MTZ(sibling) (n=12 larvae per group). (E) Statistical analysis of the number of MHCs from panel A-C. ****p< 0.0001; DMSO vs. MTZ, **p< 0.008; MTZ vs. MTZ(sibling) (n=12 larvae per group).

Figure 3.

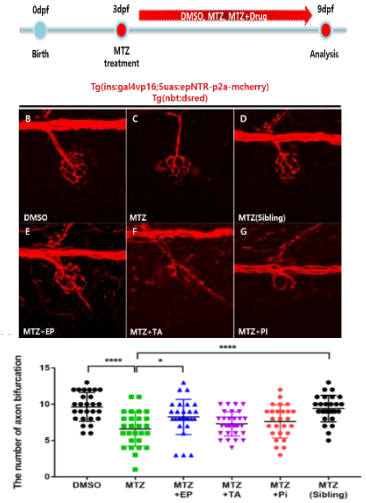


Figure 3. Epalestat (EP) prevented the defects of sensory nerves of the lateral line in zebrafish peripheral nervous system (PNS), but thiotic acid (TA) and pirenzepine (PI) did not.

A. Schematic timetable of chemical treatment for the prevention test of DPN drugs. **B-G.** Nerve endings of the lateral line in *tg(ins:gal4vp16);tg(5xuas:epNTR-p2a-mCherry);tg(nbt;dsred)* zebrafish PNS. **H.** Quantification of the number of axon bifurcation in zebrafish lateral line.

CONCLUSION

Among TA, EP, and PI in the DPN drugs, EP solely have prevention effects on the hyperglycemia-induced peripheral neuropathy in the transgenic zebrafish