Decreasing Apoptosis in Diabetic Retina with Subconjunctivally Implanted Hydrogels: One Step toward Potential Treatment of Diabetic Retinopathy

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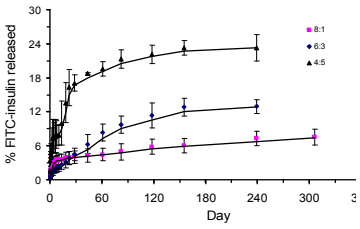
Statement of Purpose: Diabetic retinopathy is a leading cause of blindness in diabetic patients that involves early onset retinal neuronal cell loss. Our earlier works have demonstrated that intensive systemic insulin therapy reduces retinal cell death in diabetic rats, and insulin rescues retinal neuronal cells from apoptotic cell death. We have designed subconjunctivally implantable hydrogels for long-term delivery of low doses of insulin to the retina for treating diabetic retinopathy.

Methods: Hydrogels consisting of thermoresponsive and hydrolytically degradable properties were tailored for long-term release of low doses of insulin. The hydrogels were synthesized by UV photopolymerization of prepolymer solution in dimethyl formamide. FITC-labeled insulin or Humalog was loaded during the synthesis process. FITC-labeled insulin from the hydrogels was released in phosphate buffered saline, PBS, at 37 °C and was monitored by measuring fluorescence intensity. Hydrogels loaded with FITC-labeled insulin or Humalog® were implanted subconjunctivally in normal rats. Confocal microscopy was used to detect the presence of FITC-labeled insulin in the retina. Humalog-specific radioimmunoassay (RIA) was used to detect the Humalog® in the retina. Ex-vivo retinal cultures were performed to check if the insulin released from hydrogels still have bio-activity. Whole retinas were treated with hydrogels loaded with Humalog® for 5 minutes. Retinal protein were extracted and used for Western blot analysis for Akt and immunoprecipitation for insulin receptor beta (IRß) and insulin-like growth factor receptor (IGF1R) of released Humalog was tested by Akt and IRß, and IGF1R tyrosine phosphorylations in the retina ex vivo. Streptozotocin induced 8 weeks diabetic rats were treated with subconjunctival implantation of the Humalog® loaded hydrogels. Retinas were extracted 2 day or 1 week later and used for cell death assay.

Results: The designed hydrogels released low doses of FITC-labeled insulin for more than 5 months in phosphate buffered saline at 37° C (Fig.1).



Hydrogels (2 mm diameter and 1.6 mm thick) with 8:1, 6:3, and 4:5 feeding weight ratios of NIPAAm and macromer)

Release of inulsin was modulated by composition of the hydrogels and their formulation strategies. Confocal microscopy confirmed the presence of FITC-labeled insulin after one day, one week, and one month of subconjunctival implantation. Humalog in the retina was detected by RIA post one day and one week subconjunctival implantation (Fig.2).

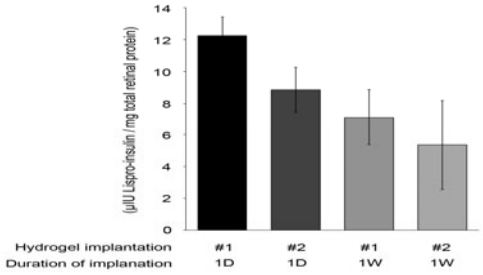


Fig.2. Humalog in the retina. #1 and # 2 contain 6.4 IU and 3.2 IU Humalog.

Phosphorylation of.Akt and tyrosine kinase of IRß, and IGF1R were increased by the treatment with hydrogel loaded with Humalog® in ex-vivo retinas. These data indicated that the released Humalog was active. Humalog®loaded hydrogel implantation decreased the DNA fragmentation in diabetic retina (Fig.3).

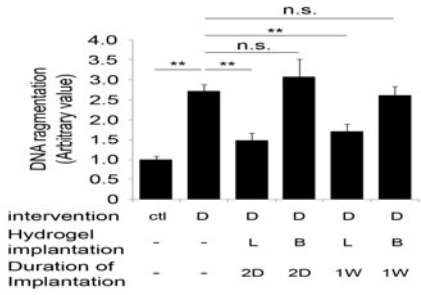


Fig.3. Streptozotocin induced 8 weeks diabetic rats were treated with the lispro-insulin loaded hydrogel (L) or blank-hydrogel (B) implantation. Retinas were extracted 2 day or 1 week later and used for cell death assay.

Conclusions: The designed hydrogels have ability to regulate insulin release kinetics and dosages, and have potential to deliver low dose insulin delivery to prevent or treat diabetic retinopathy.