Glycation of Opticin may Lead to Diabetic Retinopathy

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Introduction

Diabetic retinopathy is one of the key complications of diabetes mellitus and characterised by increased angiogenesis in the eye retina. Protein glycation and the subsequent formation of advanced glycation endproducts (AGEs) underlie the pathogenesis of diabetic complications such as retinopathy. Opticin is an extracellular matrix glycoprotein, strongly associated with the collagen fibrils of the vitreous humour . Opticin inhibits tumour-driven angiogenesis *in vivo*, growth factor-induced angiogenesis *ex vivo*, and capillary morphogenesis in three-dimensional matrices *in vitro*.

Hypothesis

To investigate glycation of opticin and whether this glycation affects its biological function with regard to angiogenesis *in vitro*.

Methods

Opticin (1 mg/ml) was glycated in 0.1 M methylglyoxal in 0.1 M sodium phosphate buffer, pH 7.4 containing 3 mM sodium azide at 37°C for up to 72 hours. Unreacted methylglyoxal was removed by dialysis. Glycation of opticin was assessed using sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) followed by silver staining and also by using matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF-MS). Bovine aortic endothelial cells (BAEC) were cultured (1.5 x 10^5 cells) in 15% foetal bovine serum for 24-hours with 25 ng/ml fibroblast growth factor-2 (FGF-2) and either 25 µg/ml glycated or native opticin. Cell migration of endothelial cells was assessed using a wound healing assay .

Results

Glycation of opticin increased formation of AGEs as period of incubation with methylglyoxal was increased (Figure 1). Glycation of opticin was also assessed using MALDI-TOF MS. There was a shift in mass as the period of incubation of opticin with methylglyoxal was increased (Figure 2). Incubation of BAEC with 25 µg/ml glycated opticin (GOPT) is increasing their cell migration as shown in (Figure 3).

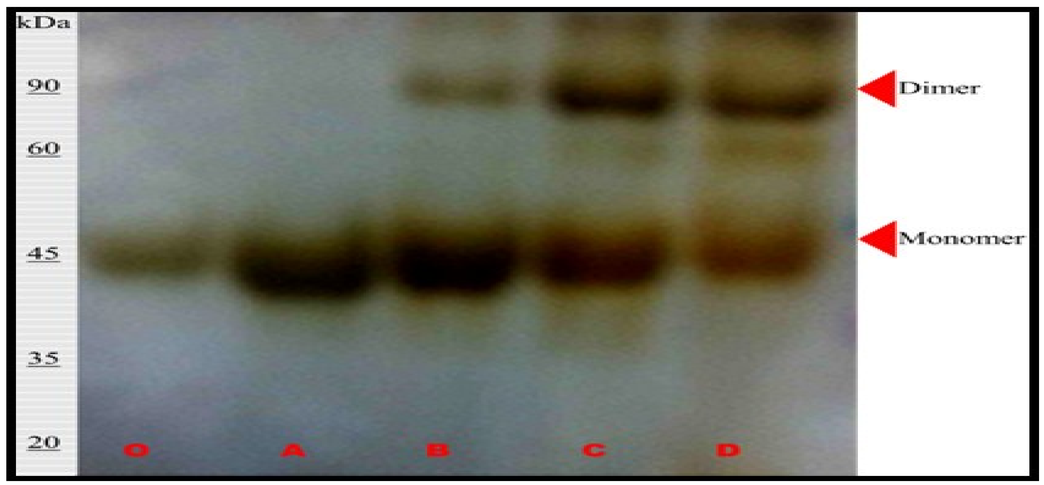
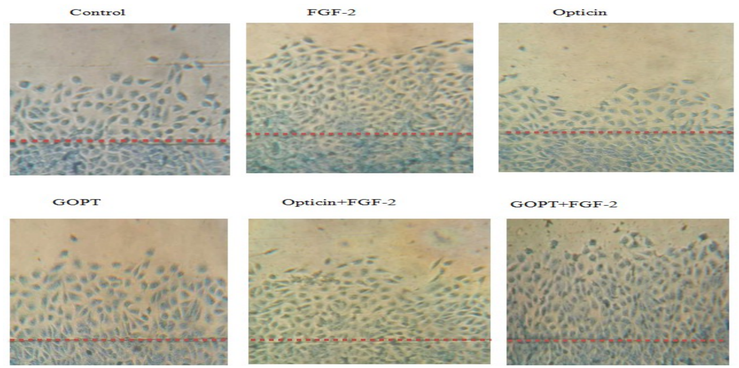


Figure1: The effect of different periods of incubation on formation of crosslinked AGEs following glycation of opticin by methylglyoxal, O:opticin, A:zero day, B:30 min, C:24 days, and D:72 days.

Figure 2: MALDI spectra of opticin glycated by methylglyoxal for different periods of incubation.

a



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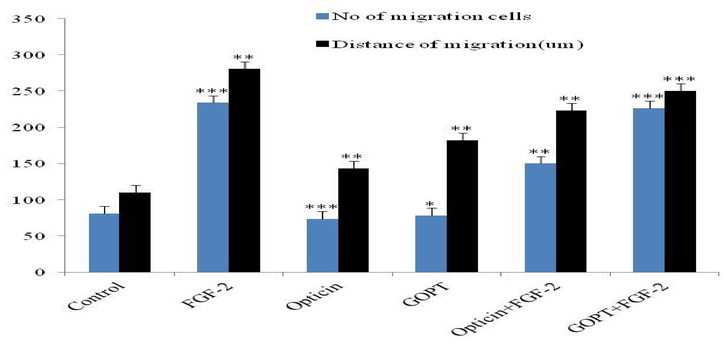

Figure 3: The effect of glycated opticin ± 25 ng/ml FGF-2 on cell migration of BAEC. (a) photo of plates after 72 hours of incubation (b) number of migration cell & distance of migration.
The bar graph shows the mean ± S.D. (*), (**), (***) signify a statistically significant difference ($p < 0.05$, $p < 0.01$, and $p<0.001$) , (n=3).

Conclusion

Glycated opticin loses its anti-angiogenic ability and this may be important in the pathogenesis of diabetic retinopathy. Future studies are required to investigate glycation of opticin *in vivo* and its role in retinopathy.