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VEGF is Involved in bFGF- Induced Murine Corneal Neovascularization

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Abstract Purpose: The purpose of this study is to determine the time and pattern of VEGF and CD-31 immunolocalization during active neovascularization in murine corneas after basic Fibroblast Growth Factor (bFGF) pellet implantation . **Methods:** Uniformly sized hydron pellets containing 80ng of bFGF and sucralfate, and blank pellets were surgically implanted in wild type C57BL/6 mice. The corneas were observed and photographed at 6 hours, 1, 4, 7, 10, 14 & 21 days post implantation and the percentage of corneal surface occupied by new vessels was calculated using NIH image program after correcting for parallax. Wild-type mouse corneas implanted with blank and bFGF containing pellets were harvested at 6 hours, 1, 4, 7, 10, 14, and 21 days after pellet implantation. The harvested corneas were embedded in OCT and processed for immunohistochemistry using rat anti-CD-31, goat anti-mouse VEGF and anti-rabbit-Ki67p antibodies. Secondary antibodies used were Cy5-conjugated anti-rat IgG, rhodamine-conjugated anti-goat IgG and fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG. **Results:** Neovascularization of the corneal stroma began on day 3 and was sustained through day 21 following bFGF pellet implantation. This was not observed post implantation of blank pellets. The percentages of the corneal surface occupied by neovascularization following bFGF pellet implantation was 0% on day 1, 1.2% ± 1.4% on day 3, 2.2% ± 1.2% on day 4, 6.8% ± 6.8% on day 7, 9.2% ± 7.4% on day 10, 13% ± 8.5% on day 14 and 15.9% ± 10.0% on day 21 respectively. In the corneal area adjacent to the limbus, the onset of VEGF stromal immunolocalization occurred 24 hours after bFGF pellet implantation and was maintained throughout the 21 day period. CD-31 localization lagged behind by 2-3 days. In the more central zone (adjacent to the pellet), the onset of VEGF stromal immunolocalization occured at day 1 and peaked at days 4-7. The lag period of CD-31 localization in this zone was 2-5 days, which correlated with the onset of clinical corneal neovascularization. Biomicroscopic and histologic examination of bFGF induced angiogenesis was also notable for the absence of corneal edema or substantial inflammation. **Conclusion:** bFGF-induced corneal neovascularization may be mediated via a VEGF-dependent pathway.