Inhibition of corneal neovascularization by $\alpha_v$-integrin antagonists in the rat

Abstract

Background: The proliferation of vascular endothelial cells and ultimately angiogenesis is inhibited by blocking integrin-mediated cell–matrix interaction. To assess the therapeutic potential of $\alpha_v$-integrin antagonists LM609 and cRGDfV in neovascularization of the anterior segment, their inhibitory effect on angiogenesis was studied in two rat models for corneal neovascularization.

Methods: Corneal neovascularization was induced in Wistar rats ($n=51$) either by silver nitrate burns or intrastromal implantation of polymer pellets containing 400 ng of fibroblast growth factor (bFGF). Animals were treated with subcutaneous injections of a cyclic $\alpha_v$-integrin antagonist (cRGDfV, 15 mg/kg body wt) or saline twice daily. Additionally, animals received intrastromal implants containing 400 ng of bFGF together with either LM609 (mAb, anti-$\alpha_v$3) or control antibody. Four days later, the animals were killed and the percentage of the surface area covered with vessels determined using digital image analysis.

Results: Systemic treatment with cRGDfV resulted in a significant reduction of corneal vessel growth in animals with bFGF-induced corneal vascularization. In corneas with silver nitrate burns, systemic cRGDfV treatment showed no significant reduction of vascularization compared with controls. Pellets containing bFGF and LM609 mAb induced significantly less neovascularization than pellets containing bFGF and control mAb.

Conclusion: Our results suggest that in the rat cornea, $\alpha_v$3 ligation does inhibit bFGF-induced neovascularization. A chemical burn of the cornea induces angiogenesis which is not inhibited by blocking $\alpha_v$-integrins. This suggests an angiogenic pathway independent of $\alpha_v$-integrins.

Introduction

Corneal neovascularization, the growth of blood vessels into physiologically avascular corneal tissue, occurs under various clinical and experimental conditions [11]. It is often observed as part of the pathological reaction in inflammatory disease of the anterior segment, corneal trauma and as a complication of corneal transplantation. The proliferation of vascular endothelial cells is an integral step in the formation of new vessels [18]. It has been shown that the proliferation of vascular endotheli-
Cyclic peptides containing the RGD motif have been synthesized to fit in a pocket created by the interaction of the α and β subunits once the heterodimer is formed and modulate integrin function [17]. Several studies provide evidence for the importance of VNR-integrins in ocular angiogenesis [6, 7, 15]. In budding vessels, the concentration of αv and β3 integrin subunits is increased on the sprouts of new vessels [4], and the adhesion receptor integrin αvβ3 has been identified as a marker of angiogenic blood vessels in chick and man [1]. A monoclonal antibody (mAb) to the integrin αvβ3 (LM609) blocked angiogenesis in the chick chorallantoic membrane [1] and cytokine-induced rabbit corneal angiogenesis [5]. Moreover, the cyclic peptide cRGDfV blocks αvβ3-dependent angiogenesis in melanoma tumors [2] and in a model of ischemia-induced retinal neovascularization [7, 15]. In humans, corneal neovascular diseases are often associated with inflammation. It was the aim of our study to evaluate the therapeutic potential of αv antagonists in a model of inflammatory corneal neovascularization. To do this, we studied the effects of αv-antagonists on the growth of vessels after chemical cautery of the rat cornea, an injury which results in a severe inflammatory reaction.

Materials and methods

Experiments were conducted in female Wistar rats (n=51) obtained from the Federal Institute for Veterinary Medicine (Berlin Marienfelde, Germany). The rats weighed 175–210 g. They were kept at 21°C in a normal 12 h light: 12 h dark cycle and fed laboratory chow ad libitum. All procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and under observation of German federal laws.

Corneal cautery

Animals were anesthetized with pentobarbital sodium (45 mg/kg body wt, Nembutal, Sanofi Pharma, Munich, Germany) and one eye was cauterised by gently pressing the tip of a silver-nitrate applicator (Argentix, Braun Melsungen, Melsungen, Germany) on the central cornea for 5 s. Before cautery, the applicator was prepared with a scalpel so that the diameter of its flat tip was 1 mm. Residual silver nitrate was removed by gentle blotting with tissue paper and rinsing the cornea with saline solution.

Preparation of pellets

Elvax and Hydron pellets were prepared as previously described [10, 12]. Both materials were used for the experiments as each polymer has physicochemical properties making one or the other better suited for the different substances which were incorporated into the pellets. Both polymers were tested for their biocompatibility by implantation of “empty” pellets. The empty pellets did not trigger any corneal neovascularization within an observation period of 3 weeks.

Fig. 1 Separated rat cornea with typical crescent neovascularization 4 days after implantation of pellets containing 400 ng bFGF (transparent pellet is marked)

Fig. 2 Flat preparation of corneal segment with ink-filled corneal and pericorneal vasculature 4 days after corneal cautery

Incorporation of bFGF into Elvax polymer

Elvax pellets (ethylene-vinyl acetate, Dupont, Wilmington, Del, USA) were washed in absolute ethanol for 60 days. The ethanol was changed daily. A 10% casting stock solution was prepared by dissolving the pellets in methylene chloride (Sigma–Aldrich Chemie, Deisenhofen, Germany). Lyophilized bFGF (recombinant human fibroblast growth factor-basic, WAK-Chemie Medical, Bad Homburg, Germany) was suspended in the casting solution and the suspension pipetted onto sterile glass slides, resulting in a final concentration of 400 ng bFGF per pellet. The pellets were left to dry at −20°C for 24 h before implantation.

Incorporation of bFGF and mAb into Hydron polymer

A 12% casting stock solution was prepared by dissolving Hydron (poly-2-hydroxyethyl methacrylate, Sigma) in absolute ethanol. So-