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Full paper

Establishment of an abnormal vascular patterning model in the mouse retina

Akane Morita [a], Shohei Sawada [a], Asami Mori [a], Shiho Arima [b], Kenji Sakamoto [a], Tohru Nagamitsu [b], Tsutomu Nakahara [a, *]

[a] Department of Molecular Pharmacology, Kitasato University School of Pharmaceutical Sciences, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan
[b] Department of Organic Synthesis, Kitasato University School of Pharmaceutical Sciences, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

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ABSTRACT

Abnormalities in retinal blood vessels and neuronal function persist in eyes undergoing retinopathy of prematurity. In this study, we examined morphological and functional changes in retinal blood vessels and neurons in mice that had undergone short-term interruption of retinal vascular development through inhibition of vascular endothelial growth factor (VEGF) signaling. In mice treated with the VEGF receptor tyrosine kinase inhibitor KRN633 on postnatal day (P) 0 and 1, the vascular density in the retinal surface increased by P12, but development of deep retinal vascular plexus and choroidal vasculature was delayed until P14. Overall retinal morphology was mostly normal in KRN633-treated mice during the observation period (~P28), with the exception of P8 and P14. On P28, abnormalities in retinal vascular patterns were evident, but electroretinogram and retinal blood perfusion were within the normal range. Abnormal architecture of retinal vasculature disturbs retinal hemodynamics; therefore, mice treated postnatally with VEGF receptor inhibitors could serve as an animal model for studying the regulatory mechanism of local retinal blood flow and the effect of persistent abnormal retinal vascular patterns on the risk of onset of retinal ischemia.

1. Introduction

Human retinal vascularization begins around gestational age 14–16 weeks (of a 40-week term gestation) and is nearly complete at term.[1–3] The interruption of normal vascular development induces the formation of morphologically abnormal retinal vasculature and leads to retinopathy of prematurity (ROP).[4–6] ROP is a major cause of pediatric blindness.[5,7]

Unlike humans, retinal vascular development in mice starts after birth. The retinal superficial vascular plexus forms during the first postnatal week, and three planar vascular networks (superficial, intermediate, and deep) are established during the third postnatal week.[8–10] In 7-day-old mice, the vasculature covers almost the entire retinal surface, but blood vessels are highly sensitive to hyperoxia.[11,12] An exposure to high concentration (75%) of oxygen for 5 days induced retinal endothelial cell apoptosis, and led to capillary degeneration in the central retina. When mice were returned to room air on postnatal day (P) 12, the hypoxic avascular retina triggered both vascular regrowth and formation of neovascular tufts, which were maximal on P17. Although the neovascular tufts disappeared by P25,[10,13] detailed analyses revealed that morphological and functional abnormalities of retinal neurons and blood vessels were present in 8-week-old mice exposed to hyperoxia between P7 and P12.[14] These findings suggest that vascular abnormalities in neonatal life affect the morphology and function of the adult retina. However, it remains unclear whether an episode of hyperoxia/hypoxia in the earlier stages of retinal vascular development permanently alters the structure and function of retinal neurons and blood vessels.

In previous studies, we demonstrated that a 2-day treatment of newborn mice with vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitors on P0 and P1 completely blocked the onset of retinal vascularization. Following treatment, delayed vascularization occurred, and abnormal retinal vascular patterns, such as decreased numbers of arteries and veins and increased numbers of arteriovenous crossing points, were detected.

* Corresponding author. Fax: +81 3 3444 6205.
E-mail address: nakaharat@pharm.kitasato-u.ac.jp (T. Nakahara).
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Abnormal retinal vascular patterns, induced by suppressing the onset of retinal vascularization, were still observed on P14 when the delay in vascularization disappeared.[15,16] The phenotypic changes in vasculature might be attributed to the inhibition of VEGF signaling at the early stage of retinal vascular development. This study was designed to expand previous studies and examined whether preventing the onset of retinal vascularization affects intermediate and deep vascular network formation and the acquired function of retinal neurons and blood vessels in mice. For this purpose, we used KRN633, a VEGF receptor tyrosine kinase inhibitor.[17] Although KRN633 inhibits several tyrosine kinases, including platelet-derived growth factor (PDGF) receptors, in addition to VEGF receptors, the inhibitory effect of KRN633 on VEGF-mediated signals in endothelial cells was much stronger than that on PDGF-mediated signals in neonatal mice.[18]

2. Materials and methods

2.1. Animals

Adult male and female Institute of Cancer Research (ICR) mice were obtained (Charles River Laboratory, Tokyo, Japan). Female animals were placed with males, and pregnant females were then removed and placed in separate cages. Daily inspections were performed to determine the day of birth (P0).

All animal procedures were performed in accordance with the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmic and Vision Research, and the Regulations for the Care and Use of Laboratory Animals in Kitasato University used by the Institutional Animal Care and Use Committee for Kitasato University.

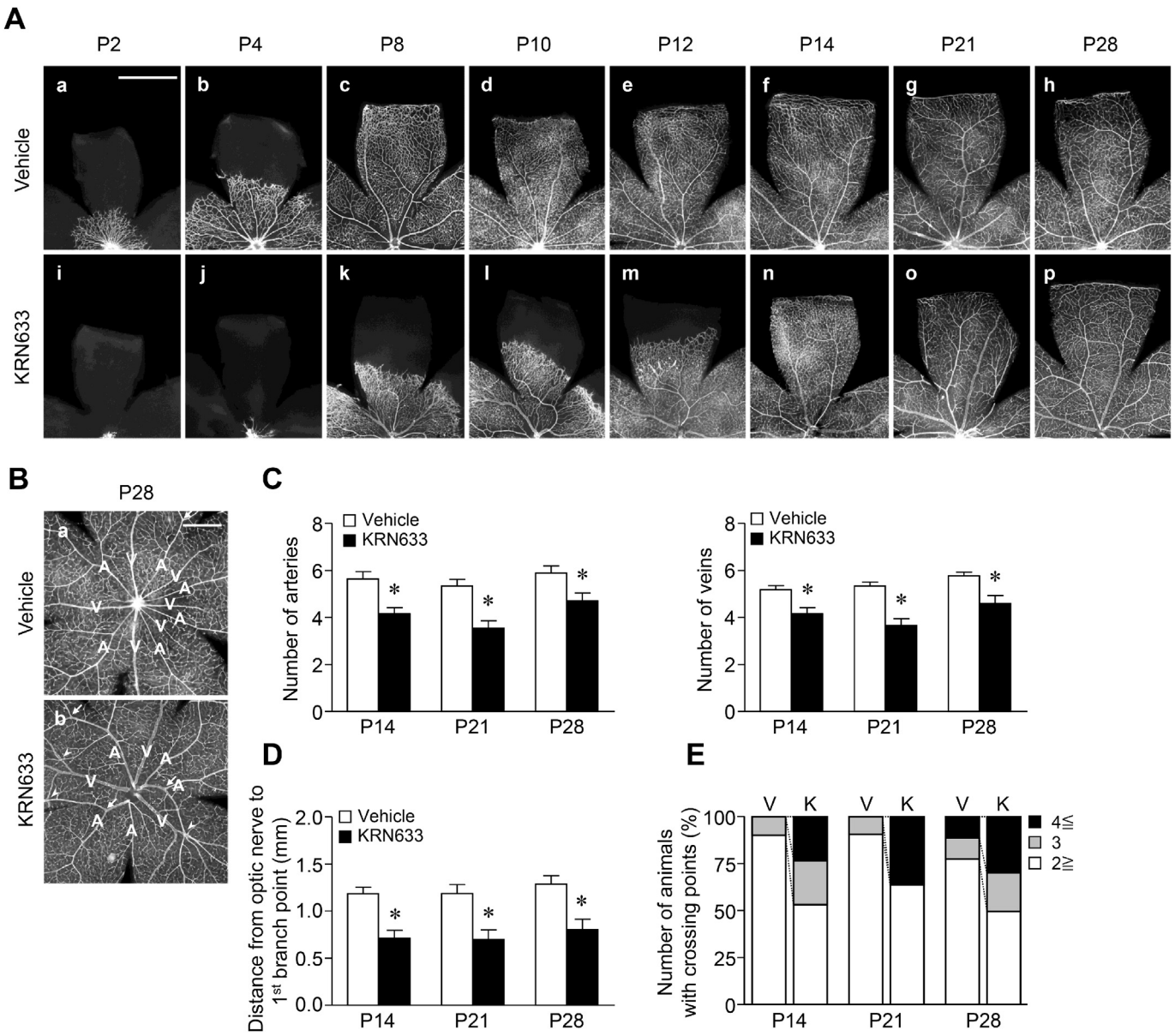

Fig. 1. Development of the superficial vasculature in the mouse retina. A & B: Fluorescence microscopy images of retinal flat-mounts stained for PECAM-1 from mice treated with vehicle (A) and KRN633 (B) on postnatal day (P) 0 and P1. Higher magnification images of the central portion in the retina of 28-day-old mice are shown in B. The "A" and "V" labels in B indicate the artery and vein, respectively. Arrows and arrowheads show the first branch points of the artery and the arteriovenous crossing points, respectively. Scale bars, 1 mm (A); 500 μm (B). C & D: Bar graphs show the numbers of arteries and veins (C) and the distance from the optic nerve head to the first branch point (D). Each column with a vertical bar represents the mean ± SE from 6 to 13 animals. $^{*}P < 0.05$ compared with vehicle-treated mice. E: Evaluation of the number of retinal arteriovenous crossing points. Animals were classified by numbers ["2≧", "3" and "4≦"] of arteriovenous crossing points seen in the retina. V: Vehicle, K: KRN633.

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