Inhibition of Experimental Choroidal Neovascularization by an Anti-growth Agent Inhibiting Vascular Endothelial Development

Yoko Matsuo, Yutao Li, Hiroyasu Taniguchi, Masanori Motoda and Tsugio Amemiya

Department of Ophthalmology and Visual Sciences, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

Purpose: To determine whether FR118487, a recently developed angiogenesis inhibitor, affects experimental choroidal neovascularization (CNV) induced by laser photocoagulation in pigmented rats.

Methods: Focal laser photocoagulation (argon green 50 mW, 0.04 seconds, 200 µm) was applied to the retinochoroid of normal Brown Norway rats. Systemic administration of FR118487 (1.0 mg/kg body weight per day) with a mini-osmotic pump implanted in the subcutaneous tissue of the neck was started just after laser photocoagulation and continued for 2 weeks. Choroidal vascular casts were made 2 weeks after laser photocoagulation and were examined with a scanning electron microscope. CNV formation was divided into three grades and evaluated.

Results: Laser-induced CNV formation was significantly less in rats given FR118487 than in control rats. CNVs in rats treated with FR118487 were less well developed than in the controls.


Key Words: Angiogenesis inhibitor, choroidal neovascularization, corrosion cast, inhibition effect, laser photocoagulation.

Introduction

Choroidal neovascularization (CNV) is common in many ocular diseases and is one of the causes of blindness in old age. Although laser photocoagulation\(^1\)–\(^4\) or submacular surgery\(^5\)–\(^8\) has been performed to treat CNV\(^3\) such therapies are not always successful, and even in successful cases, recurrence of CNV is frequent.

The effectiveness of drug therapy has also been examined and many antiangiogenic agents have been evaluated. TNP-470 is an analogue of fumagillin and inhibits corneal and choroidal neovascularization in the rat model\(^9\),\(^10\). Tranilast, an antiallergic drug, inhibits the development of experimental CNV in the rat model.\(^11\) LY333531 is a protein kinase C\(\beta\) inhibitor, which inhibits preretinal and optic nerve head neovascularization in the pig model.\(^12\) Genistein is a specific inhibitor of tyrosine kinase, which prevents regeneration of the choriocapillaris in the rabbit model.\(^13\)

FR118487, an angiogenesis inhibitor, is a chemical modification of FR111142 which was isolated from the fermentation products of *Scolecobasidium arenarium* F-2015. FR118487’s structure is (3R, 4S, 5S, 6R)-5-methoxy-4-(2R-3R)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]-1-oxaspiro[2,5]oct-6-yl-methylcarbamate.\(^14\) In the process of screening for new angiogenesis inhibitors from soil microorganisms, researchers in the Exploratory Research Laboratories of the Fujisawa Pharmaceutical Company, Osaka, found a potent new angiogenesis inhibitor, FR118487. The antiangiogenic activity of FR118487 has been reported to be 5 to 10 times stronger than that of...
FR111142. The systemic administration of FR118487 completely inhibited intravitreal neovascularization in pigmented rabbits, as shown by histopathologic examination. Angiogenesis in rabbits has also been reported to be inhibited both in vivo and in vitro by FR118487. In this study, we examined the three-dimensional features of choroidal vascular casts with a scanning electron microscope (SEM) to determine the effect of the systemic administration of FR118487 on experimental CNV in pigmented rats.

Materials and Methods

Experimental Animal Models

**CNV models.** We used 3-month-old normal Brown-Norway (BN) rats (n = 21). They were fed a standard laboratory chow diet (Oriental Kobo, Tokyo) and were given tap water ad libitum. All rats were kept in a room under controlled temperature (21 ± 2°C) and humidity (55 ± 5%) with a 12:12-hour light/dark cycle (light period, 0700–1900 hours) in the Laboratory Animal Center for Biomedical Research, Graduate School of Biomedical Sciences, Nagasaki University. The experiment was carried out in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The rats were anesthetized with intraperitoneal sodium pentobarbital anesthesia, vascular casts were cannulated and the jugular veins were cut. The vascular system was perfused with heparinized normal saline solution (500 IU/100 mL), and freshly prepared Mercox CL-2B resin (Dainippon Ink and Chemicals, Tokyo) was injected into the cannulated carotid arteries. Then the eyeballs were enucleated and left in a warm bath (56°C) for a few hours to allow polymerization and tempering of the resin. When the polymerization was complete, the ocular tissues were macerated in 20% KOH and then in 0.5% NaClO. Microdissection was done with fine tweezers and scissors under a binocular light microscope to expose the choroidal vasculature. The casts were again washed gently with running tap water and placed in 20% KOH for 1–2 days to remove residual tissues. The casts were impregnated with osmium vapor overnight, mounted on SEM stubs, and coated with ion-sputter gold palladium for examination under a Hitachi S-2360N SEM at an acceleration voltage of 10 kV.

**Observation time.** To establish the natural course of experimental CNV, corrosion casts were made 1 and 3 days, 2 weeks, and 1, 3, and 6 months after photoocoagulation; the results have already been published. CNV formation began 1 week after photoocoagulation and was complete at 1 month. According to our previous experiment, 2 weeks after photocoagulation and divided into two groups: in the control group 10 rats were given no FR118487 but only propylene glycol; in the FR118487 group 11 rats were treated with 1.0 mg/kg per day of FR118487 through an Alzet mini-osmotic pump, Model 2002 (Alza, Cupertino, CA, USA) implanted subcutaneously in the neck. Treatment started on the day of photoocoagulation and continued for 2 weeks.

**Corrosion cast and SEM.** With the rats under sodium pentobarbital anesthesia, vascular casts were made by the previously described technique. In brief, both common carotid arteries were cannulated and the jugular veins were cut. The vascular system was perfused with heparinized normal saline solution (500 IU/100 mL), and freshly prepared Mercox CL-2B resin (Dainippon Ink and Chemicals, Tokyo) was injected into the cannulated carotid arteries. Then the eyeballs were enucleated and left in a warm bath (56°C) for a few hours to allow polymerization and tempering of the resin. When the polymerization was complete, the ocular tissues were macerated in 20% KOH and then in 0.5% NaClO. Microdissection was done with fine tweezers and scissors under a binocular light microscope to expose the choroidal vasculature. The casts were again washed gently with running tap water and placed in 20% KOH for 1–2 days to remove residual tissues. The casts were impregnated with osmium vapor overnight, mounted on SEM stubs, and coated with ion-sputter gold palladium for examination under a Hitachi S-2360N SEM at an acceleration voltage of 10 kV.

Evaluation and analysis of the effects of FR118487 on experimental CNV. For evaluation of the drug treatment, architectural changes of the choriocapillaris corresponding to the laser spot were graded on corrosion casts. Laser spots were graded into three stages: stage 1 (no bud formation) has no buds and no CNV and the rough structure of the choriocapillaris persists; stage 2 (bud formation) shows vascular buds at the tip of the destroyed choriocapillaris; stage 3 (CNV formation) shows definite CNV. We excluded lesions that we could not evaluate clearly. Laser spots were evaluated in a masked manner by Drs. Taniguchi, Motoda, and Amemiya. The incidence of each stage in each group was analyzed by the Wilcoxon rank-sum test. The difference was considered significant at P < .05.

**Results**

**Overt Characteristics**

The body weight of the FR118487 group was significantly lower in both males and females than that of
the control group (Figure 1), but there was no hair loss, intestinal disturbance, or infection.

**Evaluation of FR118487**

The number of laser spots examined by vascular casts and SEM is shown in Table 1. We excluded lesions that we could not evaluate clearly and studied 59 (59.0%) of

| Table 1. Effect of FR118487 treatment of Incidence of Choroidal Neovascularization |
|---------------------------------|---------------------------------|---------------------------------|
|                                  | Control Group (%)               | FR118487 Group (%)              |
| bud(−)                           | 40 (67.8)                       | 52 (86.7)                       |
| bud(+)                           | 7 (11.9)                        | 3 (5.0)                         |
| CNV(+)                           | 12 (20.3)                       | 5 (8.3)                         |
| Total                            | n = 159 (100)                   | n = 60 (100)                    |

n = number of coagulation spots. Effects in FR118487 group are significant (Wilcoxon rank-sum test: P = 0.008).

100 laser spots in the control group and 60 (54.5%) of 110 laser spots in the FR118487 group.

Of the 59 lesions in the control group, 40 (67.8%) were stage 1, 7 (11.9%) were stage 2, and 12 (20.3%) were stage 3 (Figure 2). Of the 60 lesions in the FR118487 group, 52 (86.7%) were stage 1 (Figure 3), 3 (5.0%) were stage 2 (Figure 4), and 5 (8.3%) were stage 3 (Figure 5).

The incidence of stage 1 was significantly higher in the FR118487 group (Figure 2) than in the control group.

**Figure 1.** Growth curve of rats used in this study testing new anti-growth agent inhibiting vascular endothelial development (mean ± SD). ○: control group, ×: FR118487 group. Upper graph, male (control: n = 6, FR118487: n = 11), lower graph, female (control: n = 2, FR118487: n = 4).

**Figure 2.** Scanning electron micrograph of a cast of the choriocapillaris in a control rat 14 days after photocoagulation showing choroidal neovascularization (arrows). Bar = 200 µm.

**Figure 3.** Scanning electron micrograph of a cast of the choriocapillaris in a rat treated with FR118487 for 14 days after photocoagulation. The choriocapillaris is only partially repaired. The choriocapillaris network is irregular in the laser area where there are no buds or choroidal neovascularization. The asterisk indicates a coagulated lesion. Bar = 200 µm.
Neovascularization Inhibition by FR118487

Discussion

Using the corrosion cast method, many authors have described the effects of laser photocoagulation in the choroid and experimental CNV. The structure of CNV in rats treated with FR118487 was thin and rough (Figure 5) while that of the controls was thick and dense (Figure 2).

Neovascularization is a dominant feature in a variety of ocular angiogenic diseases, such as diabetic retinopathy, rubeosis iridis, neovascular glaucoma, and choroidal neovascularization. These neovascular lesions could be prevented or healed by FR118487. In the present study, CNV development was prevented by FR118487 and even the development of CNVs that could not be suppressed was impeded. This effect may be due to the inhibition of endothelial cell proliferation. The mechanism of FR118487 itself in the inhibition of choroidal angiogenesis still remains unclear, but TNP-470, one of the fumagillin family, is reported to block endothelial cell cycle progression in the late G1 phase through the activation of the p53 pathway, causing an accumulation of the G1 cyclin-dependent kinase inhibitor p21WAF1/CIP1. Thus, FR118487 may cause endothelial cell cycle inhibition similar to that mediated by p53 and p21WAF1/CIP1 in the choroid. The in vitro endothelial cell proliferation test and the chick CAM assay have shown that FR118487 has 5–10 times the antiangiogenic activity of FR-111142. In doses of 50–500 µg/pellet FR118487 also inhibits neovascularization in the rabbit cornea induced by an implant of endothelial cell growth supplement and heparin.

In BN rats treated with FR118487, no hair loss, intestinal disturbance or infection was seen. However, 1 mg/kg per day of FR118487 may be toxic in rats, because body weight gain was significantly less in the FR118487 group than in the control group. Poor body weight gain due to FR118487 has been reported to be reversed when this drug is discontinued. However, at the dose of 1 mg/kg per day no rats died. Further studies are needed to determine the appropriate dose. In addition, there are probably differences between laser-induced experimental CNV and

Figure 4. Scanning electron micrograph of a cast of the choriocapillaris in a rat treated with FR118487 for 14 days after photocoagulation showing small vascular buds (arrow). Bar = 200 µm.

Figure 5. Scanning electron micrograph of a cast of the choriocapillaris in a rat treated with FR118487 for 14 days after photocoagulation showing choroidal neovascularization (CNV) (arrows). However, the structure of the CNVs looks thin and rough, while that of the controls is thick and dense (Figure 4). Bar = 200 µm.

(P = .008). The structure of CNV in rats treated with FR118487 was thin and rough (Figure 5) while that of the controls was thick and dense (Figure 2).
human CNV. In conclusion, FR118487 may be a safe and promising inhibitor of human CNV formation.

The authors express their gratitude to Professor Yoshisada Shibata, Department of Radiation, Epidemiology, Radiation Effect Research Unit, Atomic Bomb Disease Institute, Nagasaki University, for analyzing the statistical data. We also thank the Fujisawa Pharmaceutical Company, Osaka, for the kind gift of FR118487.

References