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

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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.

Conclusion: *FR118487* inhibits the development of experimental CNV induced by photocoagulation in pigmented rats. **Jpn J Ophthalmol 2003;47:454–458** © 2003 Japanese Ophthalmological Society

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 Cβ 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

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FR111142.[14] The systemic administration of *FR118487* completely inhibited intravitreal neovascularization in pigmented rabbits, as shown by histopathologic examination.[15] Angiogenesis in rabbits has also been reported to be inhibited both in vivo and in vitro by *FR118487*.[14] 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 ± 2ºC) 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 (25 mg/kg), and the pupils were dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride eye drops. Argon laser (Tomey MDS10, Nagoya) was applied to the fundus under the following conditions: wavelength 540 nm, spot size 200 μm, power 50 mW, exposure time 0.04 seconds. Five laser photocoagulations were performed in each eye between the major retinal vessels of the superior retina.[16]

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 photocoagulation; the results have already been published.[16] CNV formation began 1 week after photocoagulation and was complete at 1 month. According to our previous experiment, 2 weeks after photocoagulation, the damaged choriocapillaris shows two stages of CNV formation; it was just beginning in some spots but was already completed in others. Thus, it is possible to evaluate the laser spots rather easily as they clearly represent the two stages of CNV. Consequently, we decided that 2 weeks after photocoagulation was a suitable time to evaluate the effect of *FR118487* on experimental CNV by the corrosion cast method.

Drug models. *FR118487* was supplied by Fujisawa Pharmaceutical and was dissolved in propylene glycol in our laboratory. A total of 21 BN rats were treated with laser 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 photocoagulation 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.[17] 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 (56ºC) 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-spatter 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

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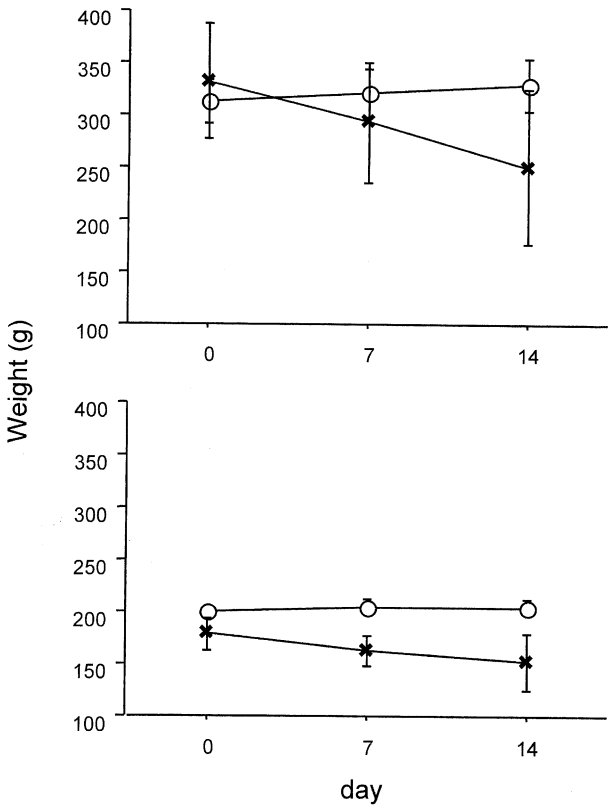

Figure 1. Growth curve of rats used in this study testing new anti-growth agent inhibiting vascular endothelial development (mean ± SD). ○: control group, ×: *FR118487* group. Upper graph, male (control: n = 6, *FR118487*: n = 11), lower graph, female (control: n = 2, *FR118487*: n = 4).

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 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

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: $P = 0.008$).

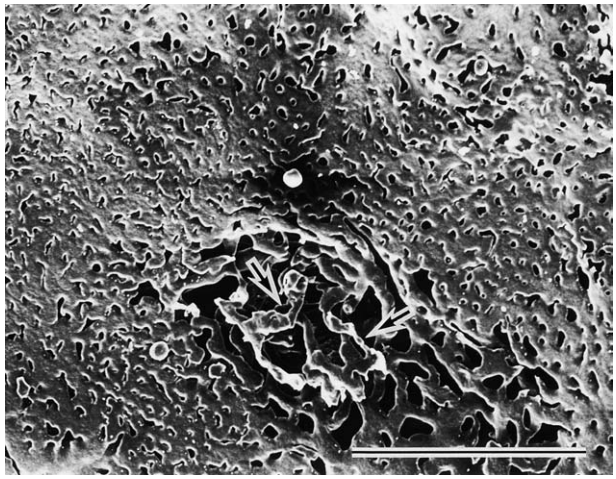

Figure 2. Scanning electron micrograph of a cast of the choriocapillaris in a control rat 14 days after photocoagulation showing choroidal neovascularization (arrows). Bar = 200 μm.

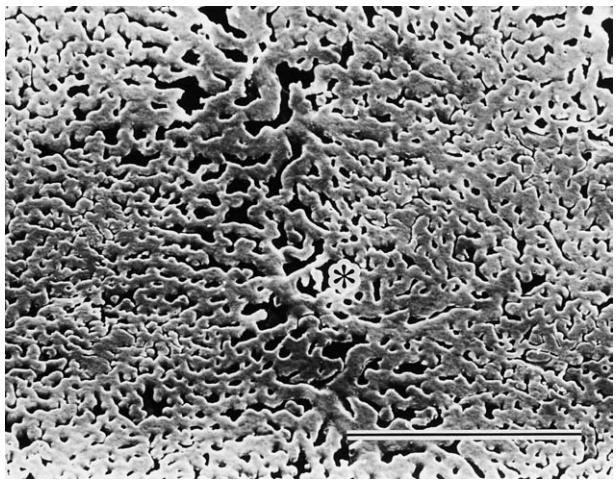

Figure 3. Scanning electron micrograph of a cast of the choriocapillaris in a rat treated with *FR118487* for 14 days after photocoagulation. The choriocapillaris is only partially repaired. The choriocapillaris network is irregular in the laser area where there are no buds or choroidal neovascularization.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

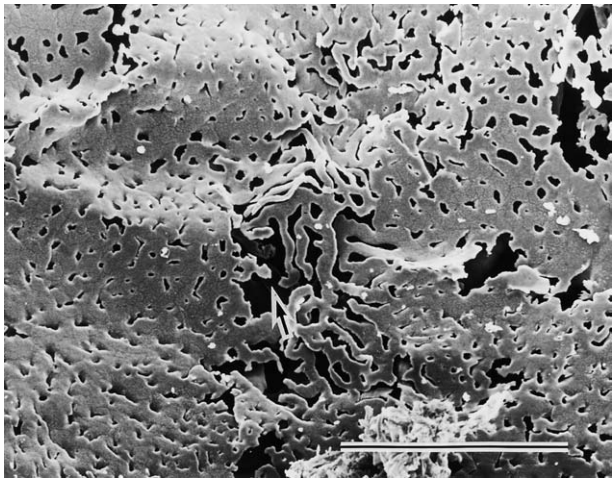

Figure 4. Scanning electron micrograph of a cast of the choriocapillaris in a rat treated with *FR118487* for 14 days after photocoagulation showing small vascular buds (arrow). Bar = 200 μm.

($P = .008$). 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).

Discussion

Using the corrosion cast method, many authors have described the effects of laser photocoagulation in the choroid and experimental CNV.[18–22] The effect of drugs on experimental CNV can be evaluated by fluorescein angiography (FA)[23–26] and indocyanine green fluorescein angiography (IA),[18] which demonstrate delicate changes in the choroidal vasculature and details of CNV formation. However, many investigators[18,19,22] have found that vascular casts can reveal more vessels and finer details than FA and IA. Although it is difficult to make choroidal casts in small animals such as mice and rats, we succeeded in making complete corrosion casts of the photocoagulated choroidal vasculature of pigmented rats.[16,17] The effect of *FR118487* has been studied widely from various aspects in vivo and in vitro in many tissues and organs.[14] It is a specific inhibitor of vascular endothelial proliferation. In this study, we have shown that *FR118487* has a significant inhibitory effect on the development of laser-induced budding and CNV. To the best of our knowledge, this is the first report of the use of this agent in choroidal neovascularization research.

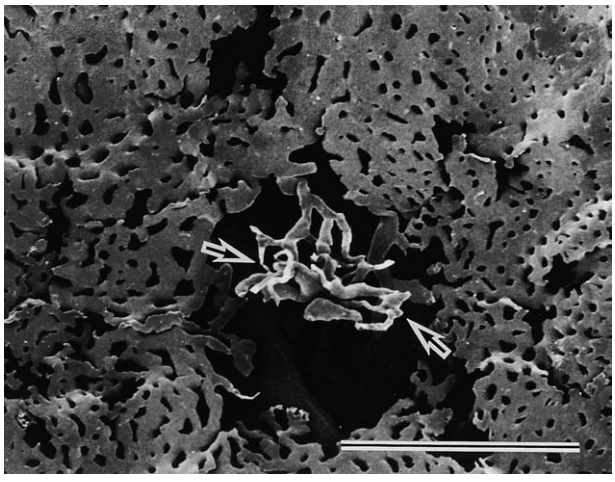

Figure 5. Scanning electron micrograph of a cast of the choriocapillaris in a rat treated with *FR118487* for 14 days after photocoagulation showing choroidal neovascularization (CNV) (arrows). However, the structure of the CNVs looks thin and rough, while that of the controls is thick and dense (Figure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

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.[27] 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 G_1 phase through the activation of the p53 pathway, causing an accumulation of the G_1 cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$.[28] Thus, *FR118487* may cause endothelial cell cycle inhibition similar to that mediated by p53 and $p21_{WF1/AIP1}$ in the choroid.[28] 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.[27]

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.[29] 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

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human CNV. In conclusion, *FR118487* may be a safe and promising inhibitor of human CNV formation.

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