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Short communication

The selective endothelin ET_A receptor antagonist FR139317 inhibits neointimal thickening in the rat

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Abstract

Endothelin is known as a potent mitogenic mediator. We tested the in vivo ability of FR139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methylpentanoyl] amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl]amino-3-(2-pyridyl)propionic acid), a selective antagonist of the endothelin ET_A receptor subtype, to inhibit neointimal thickening following photochemically induced injury of the endothelium of rat femoral artery. FR139317 (32 mg/kg s.c., twice a day) was administered for 3 weeks after the injury. FR139317 significantly decreased the neointimal area (76.3%) without changing the medial area. Therefore, it is suggested that endothelin may play an important role, via mainly endothelin ET_A receptors, in neointima formation in injured artery.

Keywords: Endothelin; Endothelin ET_A receptor antagonist; FR139317; Neointimal thickening; (Rat)

1. Introduction

Endothelin has been shown to be a potent mitogen for vascular smooth muscle cells in vitro (Komuro et al., 1988; Hirata et al., 1989; Bobik et al., 1990). The rank order of endothelin isopeptides in stimulating mitogenesis is endothelin-1 = endothelin-2 > endothelin-3 (Weissberg et al., 1990). In vivo studies indicate that exogenous endothelin-1 promotes neointima formation in the rat balloon injury model (Douglas and Ohlstein, 1993; Trachtenberg et al., 1993).

Endothelin receptors have been divided into two types, so-called 'ET_A' and 'ET_B'. The endothelin ET_A receptor is highly selective for endothelin-1 and endothelin-2 and is predominantly located in vascular smooth muscle cells (Sakurai et al., 1990). The mitogenic action of endothelin is inhibited by endothelin ET_A receptor antagonists in vitro (Ohlstein et al., 1992; Sogabe et al., 1993), suggesting the action is mediated via endothelin ET_A receptors. However, it was recently reported that the endothelin ET_B receptor subtype was more densely localized in the neointima and BQ-123 (cyclo-[D-Asp-L-Pro-D-Val-L-Leu-D-Trp]), a selective endothelin ET_A receptor antagonist, failed to prevent intimal hyperplasia (Azuma et al., 1994; Douglas et al., 1995).

In the present study, to clarify which, if either, endothelin receptor subtype is involved in the development of intimal hyperplasia, we examined the in vivo ability of another selective endothelin ET_A receptor antagonist, FR139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methylpentanoyl] amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl]amino-3-(2-pyridyl)propionic acid) (Sogabe et al., 1993), to reduce neointimal thickening in our newly developed rat model of femoral artery (Takiguchi et al., 1995). This model uses the photochemical reaction between rose bengal and green light to cause endothelial injury, which unlike the balloon injury model does not cause mechanical damage to the media (Matsuno et al., 1991).

2. Materials and methods

2.1. Animal model

Eighteen male Wistar rats weighing 250–280 g were anesthetized with sodium pentobarbital (50 mg/kg i.p.). The procedure to induce a transluminal thrombosis following endothelial injury in the femoral artery has been

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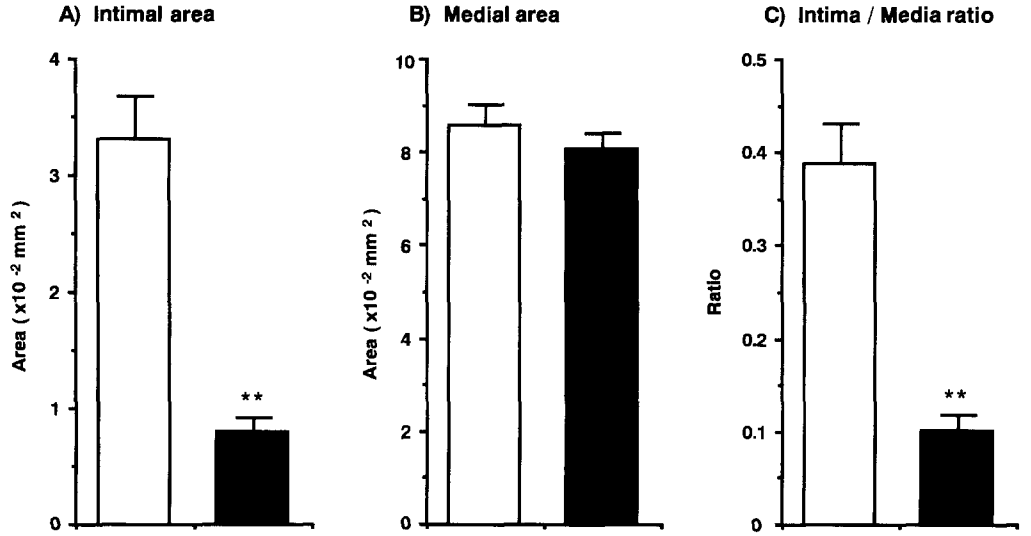

Fig. 1. Effects of FR139317 on intimal thickening in the rat femoral artery. FR139317, 32 mg/kg was administered s.c., twice a day, for 3 weeks after injury. Open columns, vehicle-treated group; closed columns, FR139317-treated group. Results are expressed as means ± S.E.M. derived from 9 animals. ** $P < 0.01$ vs. vehicle.

described in detail elsewhere (Matsuno et al., 1991). A part of the right femoral artery was carefully separated and a pulsed Doppler probe (PDV-20; Crystal Biotech America, USA) was placed on the artery. Green light (540 nm wavelength) irradiation was achieved with a L4887 irradiation apparatus (Hamamatsu Photonics, Japan). The light was directed by an optic fiber positioned about 5 mm above a segment of the femoral artery proximal to the flow probe. Under irradiation, the photosensitive dye rose bengal (Sigma, USA) was injected (15 mg/kg) via the jugular vein. Light exposure was continued until the blood flow stopped due to thrombotic occlusion of the vessel. The time required to produce occlusion was about 10 min. The thrombotic occlusion was followed by spontaneous reperfusion within the first 24 h.

The animals were then randomized to receive either FR139317 (Fujisawa, Japan) or vehicle. FR139317 was administered s.c., at 32 mg/kg, twice a day, for 3 weeks after thrombus formation. The dose is sufficient to inhibit the pressor response to endothelin-1 (Sogabe et al., 1993).

2.2. Measurement of intimal thickness

Intimal thickening was measured 3 weeks after the injury caused by thrombotic occlusion and spontaneous reflow. The irradiated (right) and non-irradiated control (left) segments of the femoral artery were fixed by infusion with 1% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate-buffered saline, pH 7.4. Then, both arteries were removed and fixed further by immersion in the same fixative. These specimens were sectioned transversely and stained with hematoxylin and eosin. The intimal and medial areas were measured by using a computer analysis system (Videoplane, Germany).

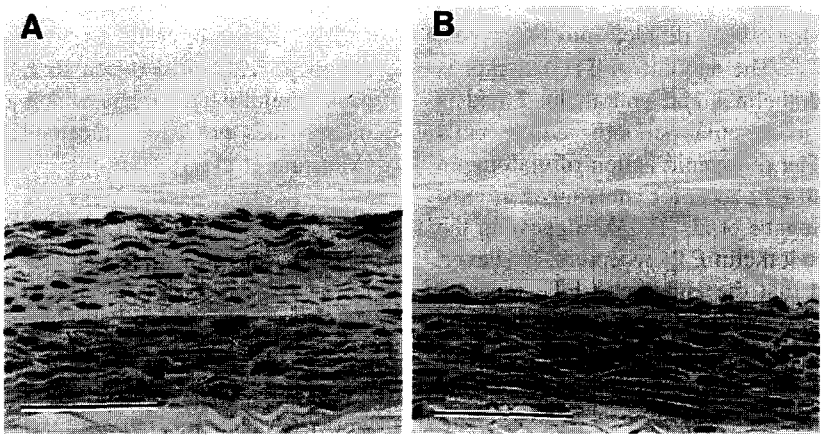

Fig. 2. Photomicrographs of representative cross-sections of injured femoral arteries at 3 weeks from rats treated with either vehicle (A) or FR139317 (B). Note marked reduction in neointimal area in FR139317-treated rats. Bar = 50 μm.

2.3. Statistical analysis

All data are expressed as the means ± S.E.M. Statistical comparisons were made with Student's *t*-test. Results were considered significantly different if $P < 0.05$.

3. Results

Intimal thickening was observed in all cases 3 weeks after the injury whereas the medial area of the irradiated artery was not different from that of the non-irradiated contralateral artery, as we previously reported (Takiguchi et al., 1995).

The effects of FR139317 on the intimal and medial areas in the rat femoral artery are summarized in Fig. 1. Administration of FR139317 (32 mg/kg s.c., twice a day) significantly decreased the intimal area (76.3%), without affecting the medial area, as compared with the vehicle control group. Therefore, the ratio of intimal to medial area was significantly reduced (74.5%) in the FR139317-treated group. Representative histological observations are shown in Fig. 2.

The treatment with FR139317 did not affect body weight and blood pressure, as previously reported (Nakamura et al., 1995).

4. Discussion

The present in vivo pharmacological study demonstrated that the selective endothelin ET_A receptor antagonist, FR139317, significantly inhibited vascular intimal thickening, suggesting a major contribution of endothelin via endothelin ET_A receptors to neointima formation after endothelial injury. This effect was unrelated to a systemic action of the drug in the dosage regimen employed.

Endothelin-1 has been shown to be a potent mitogen for vascular smooth muscle cells in vitro (Komuro et al., 1988; Hirata et al., 1989; Bobik et al., 1990). The action was completely inhibited by the selective endothelin ET_A receptor antagonists FR139317 (Sogabe et al., 1993) and BQ-123 (Ohlstein et al., 1992), which is in agreement with the fact that vascular smooth muscle cells possess predominantly endothelin ET_A receptors (Sakurai et al., 1990). Azuma et al. (1994) reported the increased immunoreactivity of endothelin-1 at the site of endothelium denudation in the rabbit balloon model. Perhaps, subsequent to denudation, the smooth muscle cells may act as an additional source of endothelin-1 present in the blood vessel wall (Resink et al., 1990). In the present study FR139317 significantly inhibited neointimal thickening (76.3%). These results strongly suggest the major contribution of endothelin to neointima formation after endothelial injury, an effect which may be mediated by endothelin ET_A receptors.

However, it has been reported that BQ-123 fails to inhibit neointimal thickening in the balloon model (Azuma et al., 1994; Douglas et al., 1995). The failure was considered to be because endothelin ET_B and non-ET_A receptor (defined as being insensitive to BQ-123) subtypes are more densely located in the neointima. The causes for the discrepancy might depend on the difference in model. However, it has not yet been reported that endothelin ET_B receptor agonists such as sarafotoxin S6C and IRL1620 induce the proliferation of vascular smooth muscle cells and that ET_B specific antagonists inhibit neointima formation in certain animal models. It has been also reported that FR139317 inhibits ET_A receptor-mediated myometrial contraction elicited by endothelin-1, but BQ-123 does not (Bacon et al., 1995).

In conclusion, the present study indicates that FR139317 is effective in preventing vascular hyperplasia after injury. Endothelin may play an important role, via mainly endothelin ET_A receptors, in neointima formation.

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