**Endothelial Hypertrophy in Acute Phase of Arteritis Induced by Fenoldopam, a Vasodilator, in Rats**

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**Abstract:** Fenoldopam, a dopaminergic (DA₁) agonist, has been known to induce arteritis in the splanchnic arteries in rats by intravenous infusion. The arteritis was characterized by medial necrosis, hemorrhage, and endothelial hyperplasia in the acute phase and subsequent inflammatory responses in the adventitia. In the present study, the hypertrophic endothelial cells were examined immunohistochemically and electronmicroscopically. Immunohistochemical examination revealed marked positive reactions of von Wilbrand factor (vWF) and factor VIII in the endothelial cells. Electronmicroscopically, the number of rough endoplasmic reticulum (rER) and the size of the nucleolus were increased. These results suggest that endothelial hypertrophy in the acute phase of fenoldopam-induced arteritis is associated with increased protein synthesis of vWF and factor VIII in response to medial necrosis and hemorrhage. (J Toxicol Pathol 2002; 15: 119–122)

**Key words:** arteritis, rat, endothelium, hypertrophy, fenoldopam, vasodilator

Fenoldopam (6-chloro-2,3,4,5-tetrahydro-1-[4-hydroxyphenyl]-1H-3-benzazepine-7,8-diol) is a selective, peripheral dopaminergic (DA₁) agonist and a potent vasodilator in rats, dogs, monkeys, and humans¹⁻⁵. It has been suggested that the vasodilative activity of fenoldopam is caused by an increase in cellular cyclic AMP through its interaction with DA₁ receptors⁶⁻⁷. Fenoldopam is also known, when given intravenously or subcutaneously, to induce arteritis in rats, but not in dogs⁸⁻¹¹. The arteritis is restricted to the muscular arteries with a large caliber (100–800 µm) in the splanchnic organs or tissues such as mesenteric, pancreatic, renal and testicular arteries, characterized by medial necrosis and hemorrhage in the acute phase and the subsequent inflammatory responses in the adventitia⁸⁻¹¹. In addition, endothelial hypertrophy was observed in the acute phase of arteritis in our previous study¹². Therefore, in the present study, the hypertrophic endothelial cells were examined immunohistochemically and electronmicroscopically.

Concerning the animal welfare, the protocols of this study have been approved by The Committee of Laboratory Animal Experimentation, Safety Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd.

Eighteen, 8-week-old F344 male rats were obtained from Charles River Japan Inc. (Kanagawa) and acclimated for a week before treatment. They were placed in hanging stainless steel wire-bottomed cages (25W × 42L × 22H cm) (2 or 3 per cage) in an animal room under controlled conditions (temperature: 23 ± 3°C, humidity: 55 ± 10%, lighting: 13 hr (8:00–21:00), and ventilation: 20 times an hour) and fed pelleted diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo) and tap water ad libitum.

Fenoldopam was obtained from Yamanouchi Pharmaceutical Co., Ltd. (Ibaraki, Japan). The purity of the compound determined by high-performance liquid chromatography (HPLC) was 99.8%. It was dissolved in physiologic saline just before intravenous infusion.

Fenoldopam was continuously administered at 6 mg/kg/hr with a rate of 0.4 mL/hr for 24 hr via the tail vein through a polyethylene catheter connected to a plastic syringe mounted on an infusion pump (STC-525, Terumo Corp., Tokyo, Japan). The rats were allowed access to food and water throughout the experimental period. Three fenoldopam-infused rats were sacrificed at the end of infusion and at days 1, 3, and 7 post-infusion (DPI), respectively. In addition, two physiologic saline-infused rats were sacrificed at the end of infusion and at 1 and 3 DPI.
respectively. At necropsy, the rats were perfused through the left ventricle with 0.01 M phosphate-buffered saline under ether anesthesia. The mesenteric and pancreatic arteries were examined with a dissecting microscope, and some of the typical lesions in these tissues were collected for electronmicroscopy. The remaining tissues were fixed by perfusion for 15 min with cold (4°C) periodate-lysine-paraformaldehyde (PLP) fixative.

In addition to perfusion with PLP fixative, the mesenteric and pancreatic arteries were further fixed with cold PLP fixative for 12 hr. The tissues were embedded in paraffin, sectioned at a thickness of 4 µm and stained with hematoxylin and eosin (H&E) and Masson’s trichrome stain for light microscopic observation. For immunohistochemistry, sections were incubated with rabbit anti-human von Willebrand Factor (vWF) (dilution 1/200, DAKO corp., California, U.S.A.) or rabbit anti-Factor VIII (dilution 1/200, Biomedca Corp., California, U.S.A.). Subsequently, they were incubated with biotinylated secondary antibodies, followed by labeled streptavidin-biotin-peroxidase complex (LSAB). They were visualized by diaminobenzidine (DAB).

At necropsy, hemorrhage and adventitial thickening were observed in arteries of the mesentery and pancreas until 7 DPI and at 3 and 5 DPI, respectively.

In histopathological examination of the mesenteric and pancreatic arteries, segmental medial necrosis, subsequent loss of medial smooth muscle cells, and hemorrhage in the space left after the loss of medial smooth muscle cells were observed at the end of infusion and at 1 DPI (Fig. 1a). Medial smooth muscle cells were hypertrophic and increased in number at 3 (Fig. 1b) and 7 (Fig. 1c) DPI, although medial necrosis and hemorrhage persisted through 7 DPI. In the intima, the endothelium showed no abnormalities at the end of infusion; at 1 and 3 DPI, it was slightly swollen, but the internal elastic lamina was intact and no thrombosis was observed. Eosinophilic hyaline materials were present in the subendothelial space at 3 DPI. The endothelium returned to normal at 7 DPI. In the adventitia, slight neutrophil and mononuclear cell infiltration was present at the end of infusion and at 1 DPI. Fibroblast proliferation was marked with infiltration of mononuclear cells at 3 DPI. A marked increase in the amount of collagen fibers was noted with scattered mononuclear cells at 7 DPI.

In immunohistochemical examination, vWF and Factor VIII antigens were abundant in the cytoplasm of most hypertrophic endothelial cells at 1 (Figs. 2a, and 2d) and 3 (Figs. 2b, and 2e) DPI, but they were detected in the cytoplasm of a small number of endothelial cells at the end of infusion and at 7 DPI as well as in physiologic saline-infused controls (Figs. 2c, and 2f). These antigens were also detected in the area of medial necrosis and hemorrhage at 3 DPI, but not at 1 DPI.

In electronmicroscopical examination, an increase in the number of rough endoplasmic reticulum (rER) was observed in the cytoplasm of most hypertrophic endothelial cells at 1 (Figs. 3a, and 3b) and 3 (Figs. 3b, and 3c) DPI, but they were detected in the cytoplasm of a small number of endothelial cells at the end of infusion and 7 DPI as well as in physiologic saline-infused controls (Figs. 3c and 3d). These antigens were also detected in the area of medial necrosis and hemorrhage at 3 DPI, but not at 1 DPI.

In the present study, hypertrophic endothelial cells were examined in the acute phase of arteritis in rats induced...
by fenoldopam electronmicroscopically and immunohistochemically. Electronmicroscopically, the number of rER and the size of the nucleolus were increased in the endothelial cells. These findings suggest that an increase in protein synthesis occurred in the endothelial cells. Immunohistochemical examination revealed that marked positive reactions of vWF and Factor VIII were detected in the endothelial cells. Newsholme et al. has reported that plasma vWF values were increased at 2–24 hr in rats given a single subcutaneous injection of fenoldopam. Therefore, endothelial hypertrophy in the acute phase of fenoldopam-induced arteritis is considered to be due to increased protein synthesis of vWF and factor VIII. Positive reactions of vWF and Factor VIII were also detected in the area of medial necrosis and hemorrhage at 3 DPI. The pathogenesis of medial necrosis is thought to be
associated with changes both in the vascular tone due to their vasodilative activity\(^9\) and in the intracellular cyclic AMP level\(^8\), and vWF and factor VIII were not considered to have a direct role in the pathogenesis of medial necrosis. Further, no abnormal changes in megakaryocytes and platelets, which were important producers of vWF, were observed in the present arteritis model. Therefore, vWF and factor VIII may have been produced in response to the medial necrosis and hemorrhage. It was thought that the eosinophilic hyaline materials in the subendothelial space may have resulted from debris of erythrocytes, necrotic medial cells, and serum factors associated with the inflammatory responses in the media.

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References