Edaravone suppresses endothelial cell injury in vein grafts of a rat model

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OBJECTIVE: Postoperative intimal hyperplasia, the major cause of vein graft occlusion, is initiated by endothelial injury. Using a high-magnification scanning electron microscope (SEM), the surfaces of endothelial cells in vein grafts were examined to investigate the protective mechanism of the free radical scavenger edaravone (Radicut, Mitsubishi Tanabe Pharma Co, Japan) against endothelial injury in postoperative intimal hyperplasia.

METHODS: In 10 male Lewis rats with a mean ($\pm$ SD) weight of 495 ± 28 g, a right epigastric vein graft was interposed into the common femoral artery. Five rats received preoperative intraperitoneal administration of edaravone (3.0 mg/kg; edaravone group). The other five received an equal volume of saline preoperatively (sham operation

group). Unoperated right epigastric veins were also examined as controls. The vein grafts from each group were examined using an S-800 Hitachi SEM (Hitachi High-Technologies Co, Japan) at $\times 10,000$ to $\times 20,000$ magnification.

RESULTS: Unoperated endothelial cells had smooth surfaces. While endothelial cells in the sham operation group had very rough surfaces with platelet adhesion, endothelial cells in the edaravone group had smooth surfaces without platelet adhesion, very similar to unoperated endothelial cells.

CONCLUSION: These SEM results showed that edaravone may maintain the smooth surface of endothelial cells. The authors postulate that edaravone suppresses postoperative intimal hyperplasia by alleviating endothelial injury.

Key Words: Edaravone (Radicut); Endothelial injury; Postoperative intimal hyperplasia; Scanning electron microscope

Postoperative intimal hyperplasia, the major cause of vein graft occlusion, is initiated by endothelial injury. We have previously reported the protective mechanism of the free radical scavenger, edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one, $C_{10}H_{10}N_2O$, MW=174.2; Radicut, Mitsubishi Tanabe Pharma Co, Japan) against endothelial injury in postoperative intimal hyperplasia (1). We have also reported that endothelial cell shape was well preserved by edaravone, using a scanning electron microscope (SEM) at a low magnification ($\times 1000$) (2). This time, using an SEM at high magnification ($\times 10,000$ to $\times 20,000$), we directly examined the surface of endothelial cells in vein grafts to investigate the protective mechanism in more detail.

METHODS

Ten male Lewis rats (mean [$\pm$ SD] weight 495 ± 28 g) were divided into two groups. Five rats received preoperative intraperitoneal administration of edaravone (3.0 mg/kg; edaravone group). The other five preoperatively received an equal volume of saline (2.0 mL/kg; sham operation group). To avoid any surgical stress to the vascular endothelial cells, intraperitoneal administration was performed, rather than intravenous administration as previously described (1). At the same time, complete general anesthesia was induced with intraperitoneal administration of 30 mg/kg of sodium pentobarbital (Nembutal, Abbott Laboratories, USA). With an operative microscope (OME-J&N J73507R, Olympus, Japan), the right epigastric

vein graft was interposed into the common femoral artery with 10-0 interrupted sutures, as previously described (1-2).

One hour after the interposition bypass, all rats were subjected to SEM study. Three unoperated right epigastric veins were also examined as controls for SEM (unoperated vein group). Specimens were prefixed in 2.5% glutaraldehyde, and postfixed in 1% osmium tetroxide. After dehydration in ethanol, they were dried at the critical point (HCP-1, Hitachi High-Technologies Co, Japan), placed on copper grids and sputter-coated with a platinum-palladium alloy. Samples were then examined with an S-800 Hitachi SEM (Hitachi High-Technologies Co, Japan) at $\times 10,000$ to $\times 20,000$ magnification.

The protocol for the present study was approved by the Hyogo College of Medicine Animal Research Committee (No 235).

RESULTS

Mean ischemic time was 65 ± 9 min and the initial patency rate was 89%. Endothelial cells in the three groups were of almost identical appearance. Representative SEM pictures are shown in Figure 1. The unoperated endothelial cells had smooth surfaces (Figure 1A). While endothelial cells in the sham operation group had very rough surfaces with platelet adhesion (Figure 1B), endothelial cells in the edaravone group had smooth surfaces without platelet adhesion, very similar to those of the unoperated endothelial cells (Figure 1C).

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Figure 1 The comparison of postoperative intimal hyperplasia in vein grafts (scanning electron microscope; original magnification $\times 10,000$). The unoperated endothelial cells had smooth surfaces (A). While endothelial cells in the sham operation group had very rough surfaces with platelet adhesion (B), the endothelial cells in the edaravone group had smooth surfaces without platelet adhesion (C), very similar to unoperated endothelial cells

DISCUSSION

In 1999, Ross (3) stated that “atherosclerosis is an inflammatory disease”. Similar to atherosclerosis, postoperative intimal hyperplasia is now also thought to be an inflammatory disease (4,5). Endothelial injury is a crucial factor in these diseases. In an electron microscopy analysis of a rat model, Stark et al (6) suggested that the regeneration of damaged endothelial cells is the first step toward postoperative intimal hyperplasia.

Using SEM at a low magnification ($\times 1000$), we previously reported that the shape of endothelial cells was well preserved by edaravone (2). However, we did not determine what happened on the surface of the endothelial cells. Our new SEM results clearly showed the rough surface endothelial injury (Figure 1B). Moreover, our new SEM results also show that edaravone maintains not only the shape but also the surface of endothelial cells (Figure 1C). Consistent with these new findings, we postulate that edaravone suppresses postoperative intimal hyperplasia by alleviating endothelial injury. However, additional research will be necessary to confirm the mechanism of how edaravone alleviates endothelial injury.

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