C-reactive Protein and Neointimal Hyperplasia in Hemodialysis PTFE Graft

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C-reactive protein is one of the inflammation markers. Plasma levels of CRP predict cardiovascular disease in normal population and ESRD patients. It has been reported that CRP is a mediator in atherogenesis and present in atherosclerotic coronary arteries. In our study, we would like to examine the expression of CRP in hemodialysis vascular access stenosis in a porcine model. Our hypothesis is that CRP is a key mediator of neointimal hyperplasia (NH) in hemodialysis PTFE graft. We actually examined if CRP is present in neointimal hyperplasia in hemodialysis arteriovenous PTFE graft, the source of CRP in the neointimal hyperplasia, and if CRP has effects on vascular smooth muscle cell (VSMC) proliferation. We made the porcine arteriovenous PTFE graft model for this study. Spiral-reinforced PTFE graft (7 cm length, 6 mm internal diameter) were placed between the carotid artery and the external jugular vein in 25-35 kg Yorkshire cross domestic pigs. The animals continuously received aspirin and clopidogrel postoperatively, and euthanized after 2-4 weeks of the operation. After euthanasia graft-vein and graft-artery anastomoses were explanted along with 2 cm of the adjacent blood vessels. They were immediately fixed in 10% buffered formalin for 24 hours. Graft and vessels were serially sectioned and embedded in paraffin. This schema shows cross section of neointimal hyperplasia. They were subjected to histological examination by H&E stain and immunohistochemistry. We could observe the H&E stain of cross section of neointimal hyperplasia in graft-vein anastomoses. As other groups have shown previously, in our pig AV PTFE graft model, moderate neointimal hyperplasia was found at 2 weeks and severe neointimal hyperplasia was found at 3-4 weeks post-operation at graft-vein anastomosis. Similar results were observed at graft-artery anastomosis.

For imunohistochemisty, anti-CRP monoclonal antibody was used. Tissue stains brown if it is positive for CRP protein. For in situ hybridization, 246 bp biotinylated anti-sense CRP RNA probe was used. Tissue stains blue if it is positive for CRP mRNA. Negative Control was biotinylated sense RNA probes. Vascular smooth muscle cells were harvested from normal porcine femoral vessels. Cells passages between 2-6 were used. The cells were cultured in a smooth muscle medium with 20% Fetal Bovine Serum. CRP (0-1 μg/mL) from human ascites was added to cells for 48 hours after 48 hours quiescent. MTT assay for cell proliferation and bromodeoxyuridine assay for DNA synthesis were performed.

These are CRP immunohistochemical staining of normal jugular vein and carotid artery. Brown is positive with CRP. Compared with normal jugular vein and carotid artery. There was intense staining for CRP on the smooth muscle cells in the neointimal hyperplastic lesions in both

graft-vein and graft-artery anastomoses. The smooth muscle cells were identified by positive staining with anti-α-smooth muscle actin antibody. CRP protein was also confirmed using western blotting in protein extracts from stenotic tissues of both venous and arterial anastomoses. We observed that CRP is present in vascular smooth muscle cells in neointmal hyperplasia. Is CRP from exogenous source such as liver? Or is CRP produced locally in neointmal hyperplasia so as vascular smooth muscle cell? We examined mRNA of CRP in vascular smooth muscle cells by in situ hybridization. In situ hybridization can detect DNA or RNA in tissues. In situ hybridization with same place of H&E staining was performed. Blue is positive for mRNA of CRP. Using anti-sense CRP probe, venous smooth muscle cells in neointimal hyperplasia were stained positive. But using sense probe they were stained negative. These results show that venous smooth muscle cells have mRNA of CRP in their cytoplasm. In situ hybridization with same place of H&E staining was performed. Using anti-sense CRP probe, arterial smooth muscle cells in neointimal hyperplasia were stained positive. But using sense probe they were stained negative. These results show that arterial smooth muscle cells have mRNA of CRP in their cytoplasm. These results suggest that both venous and arterial smooth muscle cells in neointimal hyperplasia produce CRP by themselves. I showed that CRP were present and produced by smooth muscle cells so far. Next we studied if CRP stimulates vascular smooth muscle cells proliferation? Cell proliferation was assessed by MTT assay which measures mitochondrial activity in porcine venous SMC. CRP stimulated venous smooth muscle cells proliferation. DNA synthesis was assessed by bromodexyuridine incorporation assay in porcine venous SMC. DNA syntheses were stimulated in a dose dependent manner in venous smooth muscle cells.

Dysfunction of PTFE grafts is often due to neointimal hyperplasia at the venous anastomosis. The pathogenesis of this lesion is not completely understood. Our results demonstrated that CRP is produced by vascular smooth muscle cell in the neointimal hyperplasia associated with arteriovenous PTFE grafts in our porcine model. Inasmuch as CRP also stimulates vascular smooth muscle cell proliferation, CRP might have an important autocrine function in the pathogenesis of neointimal hyperplasia of the hemodialysis PTFE graft.

Acknowledgement

We would like to thank Salt Lake City VA animal and pathology facilities, university of Utah. We also would like to thank NIH and Department of Veterans Affairs grant for funding this research.