□외국 연수 연구발표□

# Effects of Drugs and Radiation on Arterial and Venous Smooth Muscle Cells in Culture

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Vascular access failure is a major problem in chronic hemodialysis patients, leading to substantial morbidity and medical expenses. Access failure is often due to stenosis and subsequent thrombosis at venous anastomosis as a result of myointimal hyperplasia, which is largely proliferation of vascular smooth muscle cells (SMC). Therefore, inhibiting vascular SMC proliferation appears to be a reasonable approach to prevent neointimal hyperplasia.

A number of drugs and external beam radiation have been to shown to inhibit arterial SMC proliferation. The effects of these drugs and radiation on venous SMC, however, have not been reported, even though most stenoses occur at the venous anastomosis. The aim of this study was to examine if there were differences in the susceptibility of arterial and venous SMC to the inhibitory effects of anti-proliferative drugs and radiation in culture.

## Methods

Drug experiment : Human aortic and saphenous venous SMC (Clonetics) were subcultured in a Smooth Muscle Cell Growth Medium-2 BulleKit (Clonetics) supplemented with rhu-EGF, rhu-FGF and 10% FBS. Paclitaxel (0-100 μg/mL), dipyridamole (0-100 μg/mL) or tranilast (0-300 μg/mL) was added to the cell culture. After 72 hours, MTT dye reduction assay and BrdU-labeled DNA colorimetric ELISA were performed.

Radiation experiment : Human aortic and saphenous venous SMC (Clonetics) were subcultured in a Smooth Muscle Cell Growth Medium-2 BulleKit (Clonetics). Cells were trypsinized, suspended in the medium and irradiated with a single dose of 0, 1, 5, 10, 20 and 50 Gy in a cesium irradiator at room temperature. Cells were seeded onto 96-well plates. At 2, 5, 10, 15 and 20 days post-irradiation, the cell counting, MTT dye reduction assay, [$^3$H]thymidine uptake assay and BrdU-labeled DNA colorimetric ELISA were performed.

## Results

Drug experiment : All three drugs inhibited the growth of aortic and venous SMC in a dose dependent manner. On MTT assay, the inhibitory concentrations 50% (IC50) of paclitaxel were 49.2 and 17.7 μg/mL for aortic and venous SMC each. IC50 of dipyridamole were 17.7 and 5.5 μg/mL each, and those of tranilast were 143.3 and 47.2 μg/mL each. On BrdU- labeled DNA colorimetric ELISA assay, the IC50 of paclitaxel were 35.1 and 18.6 μg/mL for aortic and venous SMC each. Those of dipyridamole were 15.8 and 12.0 μg/mL each, and those of tranilast were 63.4 and 32.6 μg/mL each.

Radiation experiment : Various assays showed that radiation (1-50 Gy) was effective in inhibiting proliferation of both human aortic and venous SMC in culture in a dose dependent manner. Different assays yield somewhat different time-dependent results between arterial and venous cells. Compared to aortic SMC, venous SMC were less susceptible to radiation in all assays. For example, at 10 days post-irradiation, 1-50 Gy radiation inhibited aortic SMC proliferation by 24-66% in contrast to venous SMC by 8-25% ($p<0.01$) in cell counting assay.

## Conclusion

These results demonstrate that all three drugs (paclitaxel, dipyridamole and tranilast) and radiation were effective, with various degrees of potency, in inhibiting proliferation of both aortic and venous SMC. Compared to human aortic SMC, venous SMC were more susceptible to the various anti-proliferative drugs less susceptible to radiation than arterial SMC. In conclusion, strategies for prevention of stenosis should take into account that lesions at the venous anastomosis of vascular access may respond differently to drugs or radiation from those in arteries, such as coronary arteries.