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# Prevention of Intimal Hyperplasia by Single-dose Pre-insertion External Radiation in Canine-vein Interposition Grafts

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***Objectives:*** *to evaluate the efficacy of single-dose pre-insertion gamma radiation of vein grafts in the prevention of intimal hyperplasia.*
***Methods:*** *femoral artery interposition grafts with internal jugular vein were inserted in 12 mongrel dogs. The animals were randomly divided into two groups. Immediately before graft replacement, jugular veins were treated with a single dose of cobalt-60 radiation at 14 Gy or received no radiation (control group). Six weeks after graft insertion, the vein grafts were pressure-perfusion fixed and harvested for the histomorphometric analysis. Quantitative data on anastomotic stenosis were calculated from Gilman parameters after cross-sectional image analysis.*
***Results:*** *vein grafts treated with radiation demonstrated significantly decreased neointima formation compared with grafts in the control group. The mean Gilman parameter for the control group was 1.09 S.E.M. 0.34 mm and for the radiotherapy group was 0.65 S.E.M. 0.23 mm* ($p<0.05$). *All vein grafts in the radiotherapy group had a decreased amount of intimal and cellular infiltration.*
***Conclusion:*** *single-dose external pre-insertion gamma radiation of vein grafts reduced the amount of intimal hyperplasia in this animal model.*

*Key Words: Vein grafts; Intimal hyperplasia; Dog; Radiotherapy.*

## Introduction

Intimal hyperplasia leading to stenosis at the site of vascular or endovascular interventions such as angioplasty, stent placement or bypass grafting remains an unsolved problem. Although vascular smooth-muscle cell hyperplasia is a form of healing process, 20–50% of cases compromise the vessel lumen with ultimate failure of the reconstruction.[1]

External beam or intravascular radiation therapy following vascular reconstructions has been shown to reduce intimal hyperplasia in several experimental and clinical studies.[2–6] One of the major concerns following the use of irradiation is the occurrence of side-effects.[7,8] The aim of the present study was to evaluate the efficacy of external gamma radiation of vein grafts before insertion with histopathologic and morphometric analysis in a canine model.

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## Material and Methods

Twelve mongrel dogs of both sexes were randomly divided into 2 groups. The vein grafts in the study group ($n=6$) received radiotherapy and the remainder were evaluated as the control group. All animals were treated in compliance with the "Guiding principles in the care and use of animals" approved by the council of the American Physiological Society (guide for the care and use of laboratory animals, 7th edn, Washington DC: Natl Acad Press, 1996). Study approval was obtained from the local Ethics Committee for animals.

Anaesthesia was induced by xylasine hydrochloride (10 mg/kg) and maintained with fentanyl (5 μg/kg). Incisions, 10 cm in length were made through the skin along each external jugular vein. External jugular vein of 8–10 cm was isolated just distal to the internal and maxillary veins and proximal to the brachiocephalic branch, with careful ligation of all tributaries. Following the closure of the lateral neck incisions, superficial femoral arteries of the animals were isolated. Each

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animal received a reversed jugular-vein interposition graft in the superficial femoral artery after excision of the artery with end-to-end anastomoses by using 6-0 prolene sutures (Ethicon, Somerville, NJ, U.S.A.). Immediately before the graft interposition, saphenous vein grafts were treated with a single dose of radiation in the study group or received no radiation (control group). External-beam radiation was performed by the Co-60 teletherapy system. The vein grafts were held in isotonic NaCl and the procedure took 15 minutes. The time delay was mimicked in the control group. The isodose was calculated and a 14 Gy to 1 cm source distance was applied. After vein-graft implantations, the wounds were closed and the dogs were returned to their cage for recovery. Each animal received a total dose of 1000 IU heparin during the operation.

Six weeks after the operation, the animals were anaesthetised and the saphenous vein grafts were pressure-perfusion fixed at mean arterial pressure and harvested for the histomorphometric analysis. The specimens were processed in the usual way for paraffin embedding. Five-micron-thick sections were stained with haematoxylin–eosin for light microscopic examination. The harvested graft segments were processed and then cut at three different levels as proximal, midgraft and distal graft segments. Quantitative data on stenosis of the vein grafts were obtained from histological slides as described in detail previously.[9] A special macro program was used to draw the lumina of the vascular segments. Then, the absolute cross-sectional area of the lumen and its circumference were determined for morphometric analyses which described the rate of luminal narrowing. This is important for the studies, especially for the grafts different in size and shape.

### *Image analysis*

Quantitative data on the progression of intimal hyperplasia were obtained from histologic slides. Photomicrographs of these slides were made at a magnification of $\times 40$ (Zeiss Axioscop, $\times 40$ planapocromat objective and Koehler illumination) for measurement of intimal hyperplasia and transferred on IBM-compatible PC (Intel 486, DX2, 66 MHz). A triple-chip colour CCD video camera and a special frame-grabber board (Kontron, Germany) were used to capture the images. After all the images were transferred to the computer, they were analysed using KS 400 version 2.0 image analysis system (Kontron, Germany). By the help of a special macro program, lumens of the vascular segments were drawn. Then, the absolute cross-sectional area of the lumen and the circumference of the lumen were determined. This information permitted the calculation of Gilman parameter for each graft segment, which described the rate of luminal narrowing.[10] The Gilman parameter was calculated as follows: $d = \Delta A/p$, where $\Delta A$ is the area of intimal thickening compared with the non-thickened graft cross-sectional area (the area within the boundary defined by the internal elastic lamina); p is the perimeter difference between the intimal thickening and the non-thickened graft; and d represents the progression of luminal narrowing measured in millimetres (mm). This information is especially important for clinical studies, as these typically involve grafts of different size and shape.

Using the absolute reduction in area of the grafts (graft 1 reduction in area $= 20.9\ mm^2$ and graft 2 reduction in area $= 7.5\ mm^2$, ratio $1/2 = 2.8$), one would conclude that graft 1 has almost three-fold greater reduction in luminal area when compared with graft 2. If we use the comparison using percentage reduction in area (graft 1 percentage reduction in area = 49% and graft 2 percentage reduction in area = 85%, ratio $2/1 = 1.7$), this analysis concludes that graft 2 has greater relative reduction in luminal area when compared with graft 1. If we compare the grafts using Gilman parameter ($d = \Delta A/p$, graft 1; $d = 0.96$ mm, graft 2; $d = 0.96$ mm), this analysis shows that the rate of luminal reduction is the same for both grafts, regardless of size and shape differences. Statistical analyses were performed by Mann–Whitney *U* and Kruskal–Wallis analysis of variance (ANOVA). $p<0.05$ was considered statistically significant. All values reported are means and standard error of the mean (S.E.M.).

## Results

All the grafts were patent at the time of removal. The mean Gilman parameters in the radiotherapy group was 0.65 S.E.M. 0.23 and 1.09 S.E.M. 0.34 in the control group representing significantly less luminal narrowing rate in the study group ($p<0.05$). The difference in proximal, medial and distal graft segments were also significant between the group as illustrated in Fig. 1 ($p<0.05$), although the proximal segments both had higher rates of stenosis compared with the medial and distal segments ($p>0.05$).

Haematoxylin-and-eosin-stained sections of all segments were examined with the light microscopy six weeks after implantation. Endothelial cells were preserved in all the specimens. There were significant

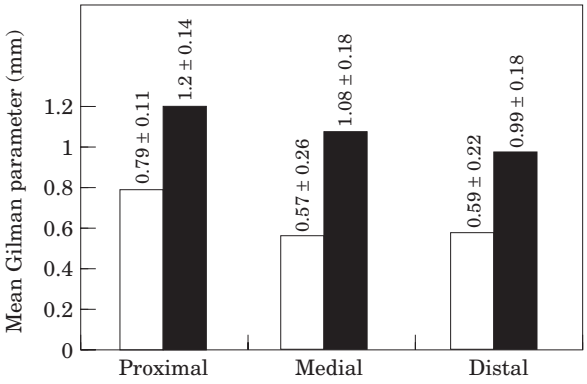


**Fig. 1.** Mean Gilman parameters from different graft segments. (□) Radiotherapy group; (■) control group.

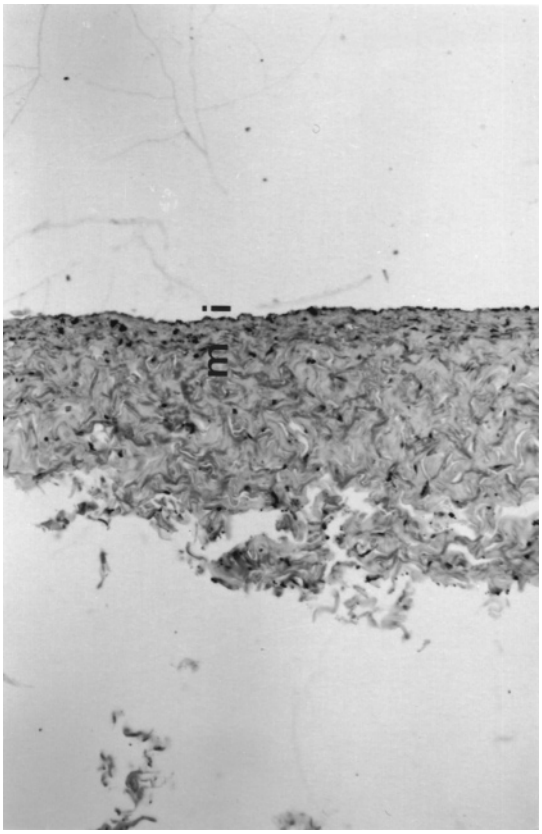


**Fig. 2.** Vein graft segment before insertion (H&E, ×200) (m: media, i: intima). Intimal and medial thickening is non-existent.


**Fig. 3.** Vein graft segment of an animal in the control group after 6 weeks (H&E, ×100) (m: media; i: intima). Highly thickened intima and media resembling intimal hyperplasia.


**Fig. 4.** Vein graft segment of an animal in the radiotherapy group after 6 weeks (H&E, ×200) (m: media, i: intima). Intimal hyperplasia is considerably less than control group.

histologic differences between the groups. All jugular vein grafts treated with radiation therapy demonstrated significantly decreased neointima formation compared with the control graft segments. They also had a decreased amount of cellular proliferation in the medial area. The markedly thickened intima and media have been observed in the control group. Additionally, there was a diffuse replacement of the media with inflammatory cellular elements. Photomicrographs of the histological specimens are shown in Figures 2, 3 and 4 at magnification ×200. Figure 2 represents a typical segment obtained before the insertion of the vein graft. The cellular infiltration throughout the vein graft and intimal hyperplasia in this figure is non-existent. In Figure 3, the highly

thickened intima and medial segments with cellular infiltration can be seen, representing the control group. In the study group, intimal hyperplasia and medial proliferation were considerably lower than the control group (Fig. 4).

## Discussion

Intimal hyperplasia represents a response to any form of vascular injury, including autologous saphenous vein grafts into the aortocoronary and the peripheral vascular circulation or after balloon angioplasty and stent insertion. In a proportion of cases it is not self-limiting, but progresses to cause stenosis in 20% of infrainguinal grafts and 40–60% of patients undergoing coronary angioplasty.[11,12] Stenoses are also observed after peripheral arterial angioplasty at anastomatic sites following bypass grafting and after carotid endarterectomy.[13–15] The process involves proliferation and migration of the smooth-muscle cells to the subintimal place.[16] Several therapeutic approaches have been attempted to curtail restenosis, but do not appear able to prevent myointimal hyperplasia in vein grafts in humans.[17–19] Heparin, which is the physiological growth inhibitor of vascular smooth-muscle cells, is also ineffective in reducing restenosis.[20]

Local radiotherapy has been used for several decades to reduce the postoperative recurrences of fibrovascular proliferations of pterygia, keloids and heterotopic bone formation after total hip replacements.[21–24] Similarly, in animal and human experiments, radiotherapy has been shown to be an effective means of preventing intimal hyperplasia.[2–6] Irradiation causes cell death by producing irreparable damage to DNA. This is believed to be the mechanism of inhibition of vascular smooth-muscle cell proliferation. Additionally, it may also alter the synthesis of growth factors that may play a role in cell proliferation or induce apoptosis.[25–28] Delivery of irradiation can be either intraluminal via a catheter or extraluminal using an external beam radiation. Both forms have been found to be effective,[29,30] but studies suggest that external forms of irradiation provide a more even dose distribution to vessel walls.[31,32] On the other hand, the advantages of intraluminal devices include the tight dose localisation and the ability to perform all interventions with a single attempt.

Friedman *et al.*[33,34] reported one of the first articles about the use of radiation to inhibit the intimal hyperplasia by using gamma radiation in an atherosclerotic rabbit model. The dose of radiation needed to reduce intimal hyperplasia varied from 3 to 20 Gy.[35,36] Weidermann *et al.*[37,38] showed a significant reduction in intimal hyperplasia at the coronary arteries in a porcine model. Furthermore, a dose response was reported with the results, using 14 Gy as the upper border.[39] Both medial and adventitial haemorrhage were observed above the doses of 20 Gy.[40] The study by Mayberg *et al.*[41] also showed that doses greater than 10 Gy had negligible effect on intimal hyperplasia. Contrary to the findings in literature, Schwartz *et al.*[35] found an increased amount of intimal hyperplasia in their model. The success of radiotherapy in animal models has more recently extended its application in clinical trials and, although several results are preliminary, initial studies have shown promising results. Schopohl *et al.*[14] demonstrated that restenosis in the femoral arteries after balloon angioplasty and stent implantation were prevented by using iridium-192 via intraluminal brachytherapy with a dose of 12 Gy. Teirstein *et al.*[5] used gamma radiation at coronary restenosis in patients undergoing percutaneous transluminal coronary angioplasty and stent replacement and showed the reduced rate of subsequent restenosis.

One of the major drawbacks following radiotherapy for the intimal hyperplasia is the possible side-effects, such as: arteritis, coronary artery stenosis, pericarditis and secondary malignancy due to its deep penetration. Aneurysmal dilatation is also an important risk in the long-term follow-up after the irradiation therapy.[42,43] Although these have been associated with doses considerably higher than in the treatment of intimal hyperplasia,[7,8] further studies analysing the long-term complications of this model are required. Using external beam radiation before the insertion of a vein graft might prevent some of these possible complications. In this study, Gilman parameters were calculated for comparative descriptions of the relative progression of intimal hyperplasia, as we described in our previous article for morphometric analysis.[9] In this study, there was a significant decrease in Gilman parameters in the radiotherapy group, which represented marked reduction of intimal hyperplasia progressing into the vascular lumen. Histological examination of grafts in the radiotherapy group also showed decreased intimal hyperplasia and medial proliferation at 6 weeks.

In conclusion, the findings from this experimental study suggested that pre-insertion, single-dose external gamma radiation of vein grafts substantially reduced the rate of subsequent intimal hyperplasia. The local application of radiation to the isolated vascular graft not only might prevent systemic side-effects of external radiation but also offers tightly controlled, even dose administration. Further studies are needed

for long-term evaluation of the early beneficial effects of this new modality.

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