



Group leader Fiona Stewart

Fiona Stewart PhD Group leader
Nicola Russell MD PhD Academic staff
Paul Baas MD PhD Academic staff
Fijs Van Leeuwen PhD Academic staff
Saske Hoving PhD Post-doc
Marion Scharpfenecker PhD Post-doc
Ingar Seemann MSc PhD student
Ben Floot Technical staff
Johannes Te Poele Technical staff
Nils Visser Technical staff

Publications

Hoving S, Heeneman S, Gijbels MJJ, Te Poele JAM, Bolla M, Pol JFC, Simons MY, Russell NS, Daemen MJ, Stewart FA. *NO-donating aspirin and aspirin partially inhibit age-related atherosclerosis but not radiation-induced atherosclerosis in ApoE null mice.* *PLoS one* 2010;5:e12874

Stewart FA, Hoving S, Russell NS. *Vascular damage as an underlying mechanism of cardiac and cerebral toxicity in irradiated cancer patients.* *Radiat Res.* 2010

Rosenfelder N, Stewart F, Brada M. *Vascular effects of radiation in the central nervous system.* In: Shrieve DC, Loeffler JS (eds). *Human Radiation Injury.* Philadelphia: Lippincott Williams & Wilkins, 2010 (in press)

RADIATION-INDUCED VASCULAR DAMAGE

Vascular damage in normal tissues is a serious late complication of cancer patients after radiotherapy. This manifests as atherosclerosis in large vessels and telangiectasia (dilated, thin-walled blood vessels prone to bleeding) and perfusion defects in capillaries. In our studies we focus on the mechanisms of development of radiation-induced vascular damage, with the ultimate goal of designing appropriate intervention strategies to inhibit or prevent this.

Radiation-induced microvascular damage and fibrosis development

(collaboration with Peter ten Dijke, LUMC, Leiden)

The mechanisms whereby telangiectasia develop are largely unclear. The vascular phenotype suggests that aberrant repair responses, including TGF- signalling, are involved. In addition to vascular injury, irradiation causes excessive connective tissue formation (fibrosis). Fibrosis develops mainly through the actions of TGF- and its downstream targets.

We used irradiated mouse kidneys (a microvascular rich organ) as a model to study the molecular mechanisms and cellular alterations leading to telangiectasia and fibrosis. These studies identified the TGF- co-receptor endoglin as being critically involved in both processes. Mice expressing reduced levels of endoglin (*Eng^{+/-}* mice) developed less radiation-induced vascular damage and less fibrosis. *Eng^{+/-}* mice showed reduced TGF- levels and decreased expression of downstream target genes related to fibrosis (*Serpine1*, *Ctgf*, and *Col3a1*). However, this was not sufficient to explain the vascular phenotype in *Eng^{+/-}* mice as vascular repair genes (*Vegfa*, *Id1*) were unaffected. We also did not measure any differences in proliferation, which might have explained improved vessel repair in irradiated *Eng^{+/-}* mice.

Further studies showed that irradiation triggered leukocytes infiltration, which was reduced in *Eng^{+/-}* mice. As leukocytes are a rich source of cytokines, chemokines and growth factors, we investigated whether these cells might influence vascular repair after irradiation. Immunohistochemical studies showed that the infiltrate mainly consisted of macrophages. Comparison of the mRNA expression profile in irradiated in wild type and *Eng^{+/-}* kidneys showed that *Eng^{+/-}* mice expressed reduced levels of the pro-inflammatory/angiogenic/fibrotic cytokines *Il6* and *Il1*. Moreover, when we studied the ability of isolated bone marrow-derived cells from wild type and *Eng^{+/-}* mice to respond to LPS stimulation, we detected impaired upregulation of *Il6* and *Il1* in cells derived from *Eng^{+/-}* mice. Staining of irradiated kidneys confirmed that these molecules are indeed produced by macrophages. These results suggest that pro-inflammatory cytokine production by macrophages contributes to the vascular and fibrotic phenotype after irradiation.

In a new project we will investigate the role of endoglin on bone-marrow-derived cell infiltration and cytokine production, versus its role in endothelial cells.

Furthermore, we will interfere with inflammatory cell infiltration and cytokine production in order to prevent erroneous repair and fibrosis development. In addition, we are aiming at stimulating proper vascular repair by promoting the recruitment of endothelial progenitor cells. We will also investigate the effects of combining anti-vascular treatments used in the clinic with radiotherapy on the development of late normal tissue damage.

Radiation induced atherosclerosis in large vessels (collaboration with Mat Daemen, Cardiovascular Research Institute, Maastricht)

We have previously shown that irradiation (1 x 8-14 Gy or 20 x 2 Gy in 4 weeks) accelerated the development of atherosclerotic plaque in carotid arteries of *ApoE^{-/-}* mice (which have elevated cholesterol levels and are prone to development of age-related atherosclerosis). Irradiation also predisposed to the formation of a vulnerable, inflammatory, thrombotic plaque phenotype with a thin fibrous cap.

Based on these results, the adhesive and thrombogenic properties of carotid endothelium were evaluated at 1-4 weeks after irradiation. High doses (14 Gy) led to an early reduction (1 week) in inflammatory markers *ICAM1* and *VCAM1*, whereas these markers have been associated with the initiation of age-related atherosclerosis. There was no change in levels of *eNOS* and *MCP1* after irradiation. High dose irradiation did cause an increase in prothrombotic tissue factor at 4

weeks, but this was counterbalanced by an increase in expression of anti-thrombotic thrombomodulin. Anti-inflammatory and anti-thrombotic drugs (aspirin and nitric oxide releasing aspirin) did not inhibit initiation or progression of radiation-induced atherosclerosis, although they did inhibit development of age-related atherosclerosis. These results would suggest that either there are other inflammatory/thrombotic mediators of radiation-induced atherosclerosis, or that other mechanisms are involved. We have now broadened our search for candidate molecules involved in triggering the radiation-induced atherosclerosis by running microarray analysis of RNA from irradiated and control arteries.

Radiation induced cardiac damage (*collaboration with Mat Daemen, Cardiovascular Research Institute, Maastricht*)

The goal of this study is to understand the functional and structural alterations of radiation-induced heart diseases, with the aim of identifying suitable intervention strategies. Male C57BL/6J mice received local heart irradiation with single doses of 2-16 Gy. Changes in cardiac function, determined by SPECT/CT and ultrasound imaging, were correlated with histo-pathological and microvascular changes at 20 to 40 weeks after irradiation. The epicardium showed inflammatory thickening, with iron-containing macrophages, from 20 weeks after ≥ 8 Gy. At 40 weeks, myocardial thickness was reduced at all radiation doses, while interstitial collagen increased after ≥ 8 Gy. Diffuse amyloid deposits, probably indicative of vascular leakage, were present in the myocardium at 40 weeks after 16 Gy. Microvascular density was decreased at 40 weeks after 16 Gy (26%). Remaining microvessels were well perfused, but there was a 50% decrease in expression of alkaline phosphatase and, after 16 Gy, a significant increase in pro-thrombotic von Willebrand Factor. Cardiac function tests showed decreased end diastolic and systolic volumes at 20 weeks after all doses (maximum 26% and 39% decrease, respectively) and increased ejection fraction (maximum 28%) after 16 Gy. There was no progression of functional damage at 40 weeks, despite progressive changes in cardiac structure. This is indicative of compensatory mechanisms to maintain cardiac function, until the damage becomes so severe that heart failure occurs.

Cardiac damage in irradiated wild type C57BL/6J mice is also being compared with atherosclerosis prone ApoE^{-/-} mice, and Eng^{+/-} mice (deficient in TGF signaling and microvascular repair). Preliminary results do not indicate more severe functional damage at 20 weeks after irradiation. However, Eng^{+/-} mice had more severe, early loss of alkaline phosphatase expression (indicative of microvascular damage) than wild type litter mates.

Angiogenic tissue response in patients with malignant mesothelioma after treatment with cisplatin, pemetrexed and Axitinib (*collaboration with Arjan Griffioen, VUMC, Amsterdam*)

Malignant pleural mesothelioma (MPM) have a high microvessel density and express increased levels of vascular endothelial growth factor receptors VEGFR₁₋₃, and other angiogenic factors like platelet-derived growth factor (PDGF). Recent studies reported a negative correlation between the microvessel density and VEGF levels in MPM biopsies, and survival. Axitinib is a potent kinase inhibitor of VEGFR₁₋₃ and PDGFR-B. A prospective, randomized, phase I/II trial has been initiated in the NKI (coordinator P Baas) to determine safety and efficacy of the addition of Axitinib to standard chemotherapy for patients with previously untreated MPM. A major limitation in evaluation of 'targeted agents', is the lack of non-invasive methods to determine efficacy in an early stage. We therefore initiated a feasibility study to investigate the effects of Axitinib on tumor vascularization. Thoracoscopic biopsies (obtained before treatment and after three treatment courses) were collected and angiogenic parameters, e.g. microvessel density, proliferating endothelial cells and expression and activation of VEGFR₂, were assessed. Moreover, plasma samples were taken during the treatment course and VEGF protein levels were determined by ELISA. Preliminary results demonstrate a reduction in vessel density and endothelial cell proliferation in the Axitinib-treated tumours. Moreover, VEGFR₂ protein expression and activity and VEGF protein levels correlated with treatment response. We are now including more patients in this study and results will be correlated with clinical chemistry data and CT or X-ray scans.