Endovascular irradiation with the liquid β-emitter Rhenium-188 to reduce restenosis after experimental wall injury

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Abstract

Objective: Postinterventional irradiation is a new therapeutic concept in the prevention of restenosis. The liquid β-emitter Rhenium-188 allows endovascular brachytherapy using a conventional balloon catheter without the problem of centering the radiation source. In an animal model of restenosis the feasibility and the dose dependent effect of intravascular brachytherapy with a Rhenium-188 filled balloon catheter was investigated. Methods: In 68 male New Zealand White rabbits after endothelial denudation of the right common carotid artery with a Fogarty catheter, endovascular irradiation was performed with a Rhenium-188 filled 3.0-mm balloon catheter using different dosages (0, 7.5, 15, 30, 45 and 60 Gy at the surface of the vessel). Then 4 weeks after the intervention the vessels were excised and histologically analyzed. Results: Whereas at 7.5 Gy the intimal area (median [first quartile; third quartile]) did not differ significantly from the control (0.46 mm² [0.33 mm², 0.75 mm²] vs. 0.49 mm² [0.34 mm², 0.66 mm²]), neointimal hyperplasia was decreased significantly at 15 Gy (0.15 mm² [0.04 mm², 0.17 mm²]) and 30 Gy (0.07 mm² [0.04 mm², 0.10 mm²]), and completely inhibited at the highest dosages (45 Gy: 0 mm² [0 mm², 0.04 mm²]; 60 Gy: 0 mm² [0 mm², 0.01 mm²]). Conclusions: Catheter transmitted endovascular irradiation with the liquid β-emitter Rhenium-188 after vascular injury is feasible and effectively reduced neointimal hyperplasia in hypercholesterolemic rabbits. A significant reduction of the neointimal formation could be found already at a radiation absorbed dose of 15 Gy at the vessel surface. Following a surface dosage of 45 Gy the proliferative response to the vessel injury is almost completely abolished. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Percutaneous transluminal coronary angioplasty (PTCA) is a safe and effective method in the treatment of coronary artery disease, but it is still limited by restenosis that occurs in 30–50% of cases within few months after the intervention. In the pathogenesis of restenosis complex mechanisms are involved: neointimal formation by migration and proliferation of vascular smooth muscle cells, extracellular matrix synthesis [1,2], and so-called negative remodeling or shrinking of the artery as a pathological response to injury of the entire vascular wall [3]. Over the past 20 years various efforts have been made to prevent restenosis by pharmacotherapy [4] or alternative interventional methods, but none of them resulted in an effective therapy concept, except stent implantation [5].

Recent experimental [6–11] and clinical studies [12–14] showed that intravascular brachytherapy might have a beneficial effect on the development of restenosis. If radiation energy is delivered to a dividing cell, the effects are independent of the source used. Cell division should be equally inhibited by γ- or β-radiation, if the energy is brought to the intended target. This aim seems to be reached most easily by using gamma sources that deeply penetrate human tissue. It has been shown in various
studies that intravascular gamma irradiation can inhibit the proliferative response after vascular injury. Waksman et al. [8] and Weinberger et al. [10] found a dose dependent decrease of neointimal formation following a dosage of 7 Gy at 2 mm from the radiation source, and of 15 Gy on the vessel surface, respectively. The radioprotection and the long application times, however, might restrict the use of gamma sources. In contrast, low ranging β-radiation can be shielded by plastic, so the radiation exposure to the staff or even the patient is minimal. The tissue penetration, however, is limited and large vessels or thick plaque material might restrict effective energy deposition. Nevertheless in first experimental trials the application of β-energy was efficient and Waksman et al. [6] even showed that at a dosage of 14 Gy at 2 mm from the radiation source, the effects of β- and γ-radiation on neointimal formation were comparable. However, the use of β-emitting wires as a radiation source is problematic: despite the use of specially designed delivery systems exact centering (essential for a homogeneous dose delivery) is almost impossible. In contrast radiotherapy with a liquid-filled (e.g. Rhenium-188) balloon catheter, that is self-centering, provides a radiation field that conforms to the vessel geometry in an optimal fashion, independent of bending of the artery, cardiac motion or stenosis morphology. Rhenium-188 is a high energy β-source in solution with a short half-life (17 h) and daily availability from a Tungsten/Rhenium generator.

The aim of this study was to examine the feasibility and the antiproliferative effect of intravascular brachytherapy after vessel injury with a Rhenium-188 filled balloon in an experimental model of restenosis.

2. Methods

2.1. Radionuclide and dosimetry

Carrier-free Rhenium-188 was obtained from a Tungsten-188/Rhenium-188 generator by elution with saline. The high-energy beta-particles ($E_{\beta_{\text{max}}}=2.12$ MeV, mean energy of 764 keV) allow therapeutic use. After perrhenate was obtained from the generator system (available from Oak Ridge National Laboratory, Oak Ridge, TN, USA), the radiotracer concentration was increased using anion-exchange columns [15].

The radiation dose emitted from a balloon catheter was measured by means of thermoluminescent dosimetry and compared to calculations using the point kernel function of Rhenium-188 [16]. High correlation was found between the theoretical and the measured radiation dose, showing a fast drop to 50% within 0.5 mm. Assuming a specific activity of 3.7 GBq/ml at the surface of a typical balloon catheter (3.0×20 mm, 135-μl volume and at 0.5-mm distance (i.e. 2-mm distance from the center of the balloon) dosages of 7.8 and 3.9 Gy/min, respectively, can be achieved [16]. So the maximum dose of 60 Gy at the vessel surface used in this study (i.e. 30 Gy at 0.5-mm depth) could be obtained within 8 min.

The volume-dose relation is explained in detail by Kotzerke et al. [16]. In short, balloon volume of a typical catheter (3.0×20 mm) increases from 0.121 ml at 3 atm to 0.151 ml at 10 atm. The balloon surface dose of a Rhenium-188 filled balloon catheter (3.0 mm at a pressure of 6 atm, 0.134-ml volume will decrease by 3.3% at 3 atm and increase by 3.9% at 10 atm for liquid Rhenium-188.

2.2. Animal model and study protocol

All experimental procedures were approved by the Animal Research Committee of the regional governmental authorities and conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

In 68 male New Zealand White rabbits under general anesthesia, the right common carotid artery was prepared. By direct atheriotomy, endothelium denudation was performed with a 2F Fogarty catheter (Baxter Healthcare, Irvine, CA, USA). Subsequently a 3.0-mm balloon catheter (Medtronic, Minneapolis, MN, USA) was introduced into the vessel and inflated with radioactive Rhenium-188 in the denuded segment. The size of the balloon catheter was chosen so that it fitted well in the vessel lumen without causing additional injury. Because of the specific activity and the intended radiation absorbed dose, the catheter had to be kept in position for energy application for ~3–10 min (Fig. 1). After this procedure the small cut in the vessel wall was sutured with 7-0 polypropylene (Ethicon, Norderstedt, Germany) and the vessel was reperfused again.

The animals were open randomized into six groups, according to the radiation dosage that was applied to the vessel wall: control (n=12), 7.5 Gy (n=8), 15 Gy (n=12), 30 Gy (n=12), 45 Gy (n=12) and 60 Gy (n=12). For feeding, a commercially available chow containing 0.2% cholesterol was chosen (Altromin, Lage, Germany). Then 4 weeks after balloon injury and irradiation the animals were sacrificed with an overdose of ketamine and xylazine. The vessels were perfusion fixated with a 2% cacodylate buffered paraformaldehyde solution for 15 min, then excised and stored in formaline for ~24 h.

2.3. Bromodeoxyuridine (BrdU) labeling

To determine the cells undergoing DNA synthesis, the thymidine analogue BrdU was given to the animals 18 and 12 h before excising the vessel. In a subcutaneous neck depot 100 mg/kg body weight BrdU and 75 mg/kg body weight deoxycytidine (both from Sigma, Deisenhofen, Germany) were applied 18 h before the animals were killed. Additionally they received 30 mg BrdU and 25 mg deoxycytidine (each per kg body weight) intramuscularly twice with 6 h between injections [17].
2.4. Histological preparation and examination

After ~24 h in formaline, the vessels were embedded in paraffin and serially cut into cross-sections of 4-μm thickness. For morphologic examinations the cuts were stained with hemalaune, hematoxylin and eosin. For morphometry Elastica van Gieson (EvG) staining was performed.

Proliferating cells, smooth muscle cells and macrophages were indicated immunohistologically, based on the biotin-avidin method [18,19].

The incorporation of BrdU during 18 h of the labeling allowed determination and quantification of cellular proliferation in the dilated arterial segment. To identify cells containing BrdU, a monoclonal antibody from Bio Cell Consulting (Greilingen, Switzerland) was used. Additionally, the cross-sections were counter-stained with hemalaune. The same indication method was used for macrophages with a RAM-11 antibody (Dako, Hamburg, Germany) and smooth muscle cells with an antibody (Renner, Dannstadt, Germany) against α-actin, which is known to be a highly specific marker of smooth muscle cells [20,21].

For quantification of the proliferating cells and macrophages in the plaques, the marked neointimal cells were counted, set in relation to the total intimal cell number and given in percent for each cell type.

To examine re-endothelialisation after intervention, endothelial cells were marked immunohistologically with an antibody against von Willebrand’s factor (vWF; anti-human VIII related antigen; Atlantic Antibodies, Incstar, Stillwater, MN, USA). The evaluation was done semi-quantitatively (control, 7.5, 30 and 60 Gy) estimating the percentage of the vWF-positive luminal cells (+: 0–25%; ++: 25–50%; +++: 50–75%; ++++: 75–100%).

Morphometrical analysis of intimal area and plaque maximum thickness was carried out with a digital image analyzer (Nikon, Düsseldorf, Germany; software package from Binlaney Consulting, Düsseldorf, Germany) that allowed an exact quantification of the intimal lesions in cross-sections after EvG-staining.

2.5. Statistical evaluation

All values are expressed as median in combination with the first and the third quartile. The significance of differences was determined using the Wilcoxon signed-rank test. Differences were considered significant at an α-error level of P<0.05.

3. Results

A total of 68 New Zealand White rabbits (3.0–3.5 kg) underwent intervention, with 12 in the control group and the others receiving intravascular brachytherapy in different dosages. Then 28 days after intervention the right common carotid artery was excised and histologically analyzed. One major problem was the high thrombosis rates in all groups (control: 50%; 7.5 Gy: 50%; 15 Gy: 42%; 30 Gy: 42%; 45 Gy: 50%; 60 Gy: 42%). The thrombosis rate, however, was comparable in all groups and independent of the radiation dosage applied. Thrombosed vessels were excluded from further analysis and do not appear in the results.

3.1. Control group

In all vessels an extended neointimal formation could be seen, containing predominantly smooth muscle cells as
Table 1  The internal elastic membrane was almost completely covered by a monolayer of endothelium-like cells (α-actin negative). The intimal area was significantly smaller than in both the control group and the group treated with 15 Gy (45 Gy: 0 mm² [0 mm², 0.04 mm²]; 60 Gy: 0 mm² [0 mm², 0.01 mm²]). Concerning the area surrounded by the internal elastic membrane (7.5 Gy: 1.80 mm² [1.58 mm², 2.01 mm²]; 15 Gy: 1.69 mm² [1.39 mm², 1.70 mm²]; 30 Gy: 1.60 mm² [1.42 mm², 1.70 mm²]; 45 Gy: 1.34 mm² [1.21 mm², 1.43 mm²]; 60 Gy: 1.36 mm² [1.26 mm², 1.63 mm²]), there was no significant difference between control and irradiated groups.

Concerning the proliferation rate in the irradiated vessels, especially at the lower doses, a trend to increased proliferative activity was obvious, but the differences were not statistically significant (7.5 Gy: 1.9% [1.6%, 2.8%]; 15 Gy: 5.7% [3.1%, 6.6%]; 30 Gy: 2.6% [2.0%, 6.3%]; 45 Gy: 5.2% [3.3%, 8.7%]; 60 Gy: 2.2% [1.8%, 3.7%]). Whereas compared to control, in low dose radiation a significantly decreased content of macrophages in the intimal layer was found (7.5 Gy: 1.1% [0.6%, 2.0%]; 15 Gy: 0.3% [0%, 2.7%]), at higher doses no difference could be seen (30 Gy: 12.0% [5.9%, 14.9%]; 45 Gy: 14.2% [3.9%, 22.0%]; 60 Gy: 2.4% [1.2%, 5.3%]).

In contrast to control groups, in all irradiated groups (independent of radiation dosage), no or only a few incoherent vWF-positive cells, as a marker for functional intact endothelium, could be detected in the luminal cell layer (Table 1).

### Semi-quantitative analysis of the von Willebrand’s Factor positive cells in the luminal cell layer as an expression of functional intact endothelium

<table>
<thead>
<tr>
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<th>Control</th>
<th>7.5 Gy</th>
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<td>+ (0–25%)</td>
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<td>++ (25–50%)</td>
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<tr>
<td>+++ (50–75%)</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>++++ (75–100%)</td>
<td>1</td>
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* Whereas in the control group 28 days after intervention re-endothelialisation was almost completed, in radiated groups only few and incoherent vWF-positive cells could be seen, independent of the applied radiation dosage.

### 3.2. Irradiation groups

Following 7.5- to 30-Gy surface dose the vessel morphology was comparable to the control group. In all histological cuts a neointimal formation of smooth muscle cells was seen (Figs. 2 and 3). Whereas in the 7.5-Gy group brachytherapy had no effect on the intimal area (0.46 mm² [0.33 mm², 0.75 mm²]), the arteries irradiated with 15 and 30 Gy had a significantly smaller neointima (15 Gy: 0.15 mm² [0.04 mm², 0.17 mm²]; 30 Gy: 0.07 mm² [0.04 mm², 0.1 mm²]). With dosages of 45 Gy or higher almost no neointimal formation could be detected.

The internal elastic membrane was almost completely covered by a monolayer of endothelium-like cells (α-actin negative). The intimal area was significantly smaller than in both the control group and the group treated with 15 Gy (45 Gy: 0 mm² [0 mm², 0.04 mm²]; 60 Gy: 0 mm² [0 mm², 0.01 mm²]). Concerning the area surrounded by the internal elastic membrane (7.5 Gy: 1.80 mm² [1.58 mm², 2.01 mm²]; 15 Gy: 1.69 mm² [1.39 mm², 1.70 mm²]; 30 Gy: 1.60 mm² [1.42 mm², 1.70 mm²]; 45 Gy: 1.34 mm² [1.21 mm², 1.43 mm²]; 60 Gy: 1.36 mm² [1.26 mm², 1.63 mm²]), there was no significant difference between control and irradiated groups.

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In contrast to control groups, in all irradiated groups (independent of radiation dosage), no or only a few incoherent vWF-positive cells, as a marker for functional intact endothelium, could be detected in the luminal cell layer (Table 1).

### 4. Discussion

The major finding of this study is the feasibility and the dose dependent effect of intravascular brachytherapy with a Rhenium-188 filled balloon on neointimal formation after vessel injury. The specific feature of this system is the simplicity of the technique despite optimal intravascular centering of the radiation source.
Fig. 3. This figure shows three histological cross-sections of a right common carotid artery 28 days after intervention. Whereas in the control group (a) an extended neointimal formation can be seen (NI), no neointima can be detected in a vessel that has been irradiated with a surface dose of 45 Gy (b). The media of the vessel is covered by a monolayer of endothelium-like cells (c).

Unfortunately, little is known about the exact absolute dose needed for efficient vascular radiotherapy or the optimal spatial and temporal dose distribution. In many successful experimental and clinical studies, the dose delivery was described poorly or inconsistently, and the area where the mentioned dosage was delivered varies. It
was recommended that dose should be specified with reference to a point 2 mm distant from the axis and the center of a catheter-based system [22]. In a radioisotope filled balloon the vessel surface represents a better reference point which is independent of the vessel diameter.

In consequence the dosages described in our experimental setting are those that have been applied to the vessel surface. Following 15-Gy surface dose (according to 7.5 Gy at 0.5-mm depth) a significant reduction of neointimal formation could be detected 4 weeks after balloon angioplasty. This result conforms with the findings of Verin et al. [11] who have also investigated the effect of 90-yttrium in a rabbit model; they found a significant reduction in percent area stenosis at their maximum dose of 18 Gy (balloon surface) after 6 weeks, while lower radiation dosages (6 and 12 Gy) were not effective.

In our study, despite the significant effect of 15 and 30 Gy, an extended neointima could still be seen in the histological cross-sections, whereas no neointimal plaques were found at 45 and 60 Gy. Waksman et al. [7] have reported the complete suppression of neointimal formation after high dose irradiation (28 and 56 Gy at 2 mm from the radiation source).

In addition to these reports, the first data about intravascular brachytherapy with Rhenium-188 in a swine model have been reported. Whereas Makkar et al. [23] found a significant effect on restenosis development in stented arteries with 14 Gy at 0.5 mm from the balloon surface, Weinberger et al. [24] showed a decrease of neointimal area with only 25 Gy, at a radiation dose of 11 Gy at 0.5-mm depth: however, no effect on neointimal formation was detected. These results conform with our data in the rabbit model, and it might be supposed that using this technique a radiation dose of at least 15 Gy at 0.5 mm from the balloon surface is required to effectively suppress intimal proliferation.

Intravascular radiotherapy, however, might not have only beneficial effects. Several severe complications such as acute thrombosis of the vessel, fibrosis or, especially in high dose brachytherapy, aneurysm formation are discussed. In accordance with Waksman’s findings [7] we did not detect structural damage in the vessel wall caused by radiation, and to a large extent the internal elastic lamina was covered by a monolayer of cells in both high dose groups. But staining for vWF revealed a problem of radiation therapy: even at the lowest radiation dosage, in contrast to control, only a few cells which were not vWF-positive could be detected in the luminal cell layer, which means the almost complete lack of functional intact endothelium 28 days after intervention. Delayed re-endothelialisation may be responsible for thrombosis of the vessel even weeks after intervention. In the porcine model, Vodovotz et al. [25] reported a dose dependent increase of the overall thrombosis rate in irradiated vessels, whereas the rate of luminal thrombus formation was found to be decreased. Thromboses of irradiated vessel segments have also been reported from human coronary brachytherapy usually occurring late after the intervention (2–3 months) which is outside the timescope of this study [26]. From our experimental data there is no evidence for higher thrombosis rates after intravascular radiation. Although we found 40–50% of the vessels thrombosed, this is more a limitation of the model (atherotomy and denudation in the same vessel) than a result of brachytherapy, as the thrombosis rate did not differ in both the control and the radiation groups. Nevertheless our study might show the causes of late thrombosis in irradiated vessels, i.e. an inhibition of re-endothelialisation even at low radiation dosages in the intimal layer.

Despite the high dosages we applied, none of the vessels showed any aneurysms or dissection, and the area surrounded by the internal elastic lamina did not differ significantly between the study groups. It is questionable whether any severe structural damage can be caused in the vessel wall by the rapid dose decrease of β-energy at the dosages used. Even in the swine model used by Waksman et al. [7], after extensive wall damage including a wide rupture of the internal elastic membrane by overstretch injury, with β-radiation of 58 Gy at 2 mm from a centered wire source, no aneurysm formation could be detected.

Fibrosis is a typical late complication after radiotherapy and normally occurs years after the exposure, so therefore it can certainly not be seen after a follow-up period of 4 weeks. Also, the exposure of the surrounding tissue using intravascular β-radiation is minimal and fibrotic changes can hardly be expected.

An unlikely but possible complication is the often discussed problem of balloon rupture in clinical application with release of radioactivity. Kotzerke et al. [27] estimated radiation exposure to 0.42 mSv/MBq Re-188 which can be reduced to 0.16 mSv/MBq by subsequent use of perchlorate blocking. Assuming a specific volume of 3.7 GBq/ml, the balloon contains 518 MBq in 0.134-ml volume. A complete release would result in a whole body radiation exposure of 83 mSv when using perchlorate. Angioplasty procedures, however, cause radiation exposures in the same range.

5. Study limitations

The hypercholesterolemic rabbit model of restenosis is established and allows an accurate measurement of all neointimal parameters, as the internal elastic lamina is not damaged. The time course of intimal proliferation after vessel injury is well known [17,28] and the results of different studies with this model could be confirmed in clinical studies [29–33]. Nevertheless this model certainly is limited in several aspects. Endothelium denudation in native vessels can hardly be compared to balloon angioplasty in a human coronary artery with extended atherosclerosis: the proliferative response after vessel injury
might differ considerably. Besides, we cannot predict the radiation dose that is needed to prevent restenosis in vessels with large atherosclerotic plaques, especially as it probably might be even higher with low range $\beta$-energy. As human plaques are often eccentric, dose delivery to the vessel wall might become inhomogeneous, resulting in decreased effect of brachytherapy (e.g. a need for higher dosages) on the one hand, and on the other, vascular radiation damage on the opposite site where the vascular wall is thinner.

A general problem of animal models is the lack of long-term results. In this study, however, in the high dose group, where neointimal hyperplasia was inhibited completely, the durability of the effect can also be expected over a longer time period.

6. Conclusions

Intravascular brachytherapy with the liquid $\beta$-emitter Rhenium-188 was easily feasible experimentally and might be also used with little effort in a normal catheter laboratory. In this rabbit model of restenosis, from a surface dose of 15 Gy neointimal formation was decreased significantly. Application of 45 Gy or more completely inhibited the vascular response to injury without any structural damage to the vessel wall.

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