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FKBP12+ S100+ Dendritic Cells as Novel Cellular Targets for Rapamycin in Post Stent Neointima

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Abstract

Background: Despite promising clinical results for rapamycin-eluting stents, the exact mechanism of action and cellular targets are not clear. Therefore, we determined the presence and spatiotemporal signal pattern of the rapamycin receptor FK506-binding protein FKBP 12 in minipig aortic segments after stent implantation.

Methods: Male minipigs underwent bare metal stent implantation to aortic segments. At days 7, 14, 30, 60 and 90 after injury, arterial cross sections were analyzed by immunohistochemistry for FKBP12 and S100+ dendritic cells.

Results: At day 7, about 25% of neointimal cells expressed FKBP12. In further time course signaling for FKBP12 decreased continuously and revealed two predilection regions at luminal and stented sites. Throughout the observation time, a significant portion of FKBP12+ cells coexpressed S100 marker.

Conclusion: The rapamycin receptor FKBP12 is predominantly present in early neointima. Colocalisation of FKBP12 and S100 suggests that dendritic cells may be another important target for rapamycin actions.

Keywords: Vascular remodelling; Rapamycin; Dendritic cells; Animal model

Introduction

The rapamycin actions of preventing in-stent restenosis seem to be complex and are still not well understood [1]. It is known that rapamycin due to formation of an intracellular complex with its receptor FKBP12 inhibits different cellular processes like proliferation of vascular smooth muscle cells, inflammation [1], protein synthesis and matrix production [2]. Moreover, rapamycin regulates the biology of dendritic cells. The last function is of a great importance as dendritic cells activate immunological responses after vascular trauma. Rapamycin may influence these cells via: (i) in vitro inhibition of interleukin-4 mediated maturing of dendritic cells [3], (ii) attenuation of antigen intake via inhibition of macropinocytosis and endocytosis [4], (iii) impairment of immunological reactions induced by dendritic cells [5], (iv) *in vivo* depletion of growth factor for dendritic cells [3] and eventually via augmentation of dendritic cell apoptosis [6].

In this context, in our present work, we sought to detect FKBP12, the primary intracellular receptor of rapamycin, in the post stent swine neointima, determine the spatiotemporal pattern of its expression as well as to evaluate the presence of FKBP12 positive neointimal dendritic cells.

Materials and Methods

Swine model of stent implantation

Male minipigs (age: 12 months, mean weight: 23.1 kg) were anesthetised by ketamine (10-15 mg/kg), atropine (1 mg), azaperone (2 mg/kg). Two days before stent implantation, the therapy with ticlopidine (250 mg once a day, p.o.) was started and it was continued in the next 4 weeks. The femoral artery was punctured percutaneously and a 9F sheath was introduced. After angiography of the descending aorta, commercially available stainless steel (American Iron und Steel Institute) 316-L-Stents (Saxx Stent, CR Bard, Tempe, AZ, USA) were implanted. The PTCA balloon (Bard Optiplast) with the stent was inflated with the pressure of 10 atmospheres within 10 sec. The balloon diameter was approximately 10-25% larger than that of the vessel at the implantation site. Periprocedurally, aspirin (250 mg i.v.) and heparin (5000 I.E. i.v.) were administrated. The animals were sacrificed with a lethal dose of pentobarbital at days 7 (n=2), 14 (n=2), 30 (n=3), 60 (n=3) and 90 (n=4). All experiments were approved by the local governmental authorities (Bezirksregierung Hannover; 42502-02/563) and complied with the NIH current guidelines (Guide for the care and use of laboratory animals; NIH publication 85-23, 1985, revised 1996).

Immunohistochemistry

Paraffin cross sections (4 µm) were dewaxed and rehydrated. After proteolysis, nonspecific antibody binding sites were inhibited with fetal calf serum. Subsequently, the sections were treated with the polyclonal rabbit anti-FKBP12 (1:500, Biomol, Hamburg, Germany) and anti-S100 (1:40, Sigma, Deisenhofen, Germany). Thereafter, AffiniPure mouse anti-rabbit IgG (1:75, Dianova Inc., Hamburg, Germany) was applied for 30 min. The visualization occurred with the APAAP method (Dianova, Hamburg, Germany) and Fast Red (Sigma, Steinheim, Germany), according to standard protocols [7,8]. Nuclei were counterstained with hematoxylin.

Double immunostaining experiments were performed analogically with the use of two chromogens: Fast Blue (Sigma, Steinheim, Germany) for FKBP12 and Fast Red for S100. In order to avoid a confusion of the blue marked nuclei with the blue stained FKBP12+ signals, the nuclei hematoxylin staining was omitted. In each experiment tissue specimens without primary antibody application served as negative controls.

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Histological analysis

The percentage of FKBP12+ or S100+ cells in neointima was determined by means of a computer-assisted morphometric system (VFG-1-grafic card/VIBAM 0.0 Software) [7,8]. In detail, FKBP12 or S100 marked cells and all neointimal cells were counted in each of five randomly selected neointimal areas per cross section. The expression of the immunostained cells was calculated as an average number of positive cells per total average number of cells within neointima for each tissue sample.

Statistical analysis

The data are expressed as mean ± SD. The differences between the means of multiple groups were compared with the Kruskal-Wallis-H test (SPSS for Windows software, version 10.0, SPSS Inc.). $P<0.05$ was considered statistically significant.

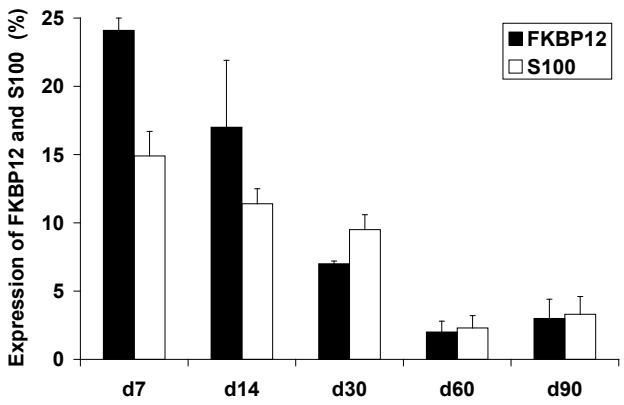

Figure 1: Average number of FKBP12 and S100 positive cells per total average number of neointimal cells presented as expression values (%) in neointima in time course after stent implantation. d=days.

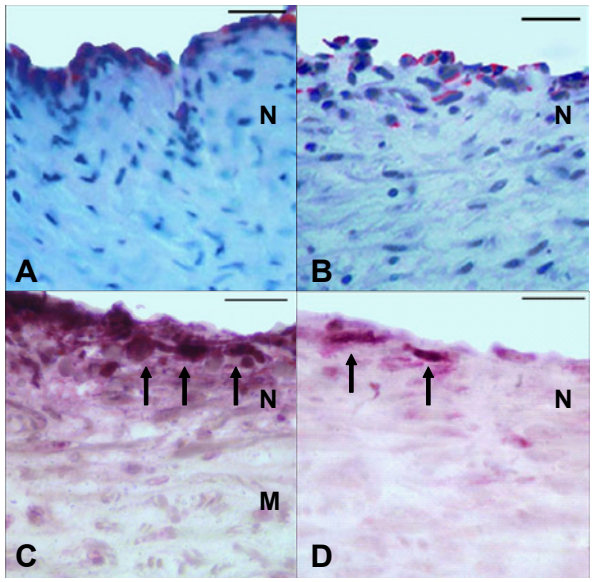

Figure 2: Luminal prevalence of FKBP12 (A) and S100 (B) signaling in neointima at day 30. Double immunostaining of FKBP12 (dark blue) and S100 (red) in neointima at days 14 (C) and 90 (D). Arrow=FKBP12+ S100+ cell. Bar=20µm. N=Neointima. M=Media.

Results

The rapamycin receptor FKBP12 was expressed in approximately 25% of all neointimal cells at day 7 after stent implantation (Figure 1). With ongoing neointima development, at day 14, the FKBP12 signaling reduced. In time course, the expression of FKBP12 further decreased and was exclusively detected in luminal areas of neointima and near to the stent struts. Mature neointima was almost free of FKBP12 signals. S100+ cells revealed a similar spatiotemporal expression pattern with two predilection regions at luminal and stented sites in late neointima (Figure 1). Differences in expression of FKBP12 and S100 were significant across the different time points studied ($p<0.05$, respectively). The double immunostaining experiments showed a colocalisation of FKBP12+ and S100+ cells throughout the observation time (Figure 2). The media and vessel-free adventitia demonstrated no signals for either of both markers at any time point. Only small adventitial vessels showed single S100 signals during the study time.

Discussion

In our study, we showed the pronounced presence of rapamycin receptor FKBP12 during early neointima formation of swine after stent implantation and the co-expression of FKBP12 and the dendritic cell marker S100. Herein, the present data extend previous findings concerning detection of FKBP12 signals in the post angioplasty neointima of rat, in human atheroma and in human in-stent restenoses [7-9].

In our work, the strongest immunolabeling for FKBP12 was detected in neointima at days 7 and 14, thus at early stages of neointima formation characterised by enhanced inflammation, cell migration, proliferation and apoptosis [10,11]; this suggests that rapamycin would be most effective in the incipient neointima. Taking into consideration the fact that rapamycin may impair reendothelialisation and promote thrombus formation [12], our data provide evidence that the time of rapamycin action should be restricted to early phases after vascular trauma. Moreover, the detection of FKBP12+ cells in peristrut neointimal regions may indicate that these cells are the primary targets for rapamycin. However, keeping in mind, the excellent lipophility of rapamycin [13], it is probable that also the luminal FKBP12+ cells are affected by rapamycin. The exposure of these cells to rapamycin influence is greater when the diffusion way is shorter, which takes place in incipient neointima. This fact supports again our hypothesis to limit the drug release time of rapamycin coated stents to early time points after vascular damage.

Another finding of our work was the identification of FKBP12+ S100+ dendritic cells as cellular targets for rapamycin action. Dendritic cells are known initiators of immunologic reactions and in consequence may contribute to neointimal growth. Several studies have shown that the function of dendritic cells may be modified by rapamycin at different levels of their maturing process [3-6]. The detection of FKBP12+ S100+ cells in post stent neointima may emphasize the antiimmune action of rapamycin in addition to its antiproliferative one underlying excellent clinical results in the prevention of restenosis via implantation of rapamycin coated stents [14].

In summary, the rapamycin receptor FKBP12 is predominantely present in early neointima after stent implantation. The FKBP12+ S100+ dendritic cells represent novel target structures for rapamycin action.

References

1. Zohlnhöfer D, Nührenberg TG, Neumann FJ, Richter T, May AE, et al. (2004) Rapamycin effects transcriptional programs in smooth muscle cells controlling proliferative and inflammatory properties. Mol Pharmacol 65: 880-889.
2. Gouëffic Y, Potter-Perigo S, Chan CK, Johnson PY, Braun K, et al. (2007) Sirolimus blocks the accumulation of hyaluronan (HA) by arterial smooth muscle cells and reduces monocyte adhesion to the ECM. Atherosclerosis 195: 23-30.
3. Hackstein H, Taner T, Zahorchak AF, Morelli AE, Logar AJ, et al. (2003) Rapamycin inhibits il-4-induced dendritic cell maturation in vitro and dendritic cell mobilization and function in vivo. Blood 101: 4457-4463.
4. Monti P, Mercalli A, Leone BE, Valerio DC, Allavena P, et al. (2003) Rapamycin impairs antigen uptake of human dendritic cells. Transplantation 75: 137-145.
5. Chiang PH, Wang L, Bonham CA, Liang X, Fung JJ, et al. (2004) Mechanistic insights into impaired dendritic cell function by rapamycin: inhibition of Jak2/Stat4 signaling pathway. J Immunol 172: 1355-1363.
6. Woltman AM, van der Kooij SW, Coffer PJ, Offringa R, Daha MR, et al. (2003) Rapamycin specifically interferes with GM-CSF signaling in human dendritic cells, leading to apoptosis via increased $p27^{KIP1}$ expression. Blood 101: 1439-1445.
7. Bauriedel G, Jabs A, Kraemer S, Nickenig G, Skowasch D (2008) Neointimal expression of rapamycin receptor FK506-binding protein FKBP12: postinjury animal and human in-stent restenosis tissue characteristics. J Vasc Res 45: 173-178.
8. Tuleta I, Bauriedel G, Steinmetz M, Pabst S, Peuster M, et al. (2010) Apoptosis-regulated survival of primarily extravascular cells in proliferative active poststent neointima. Cardiovasc Pathol 19: 353-360.
9. Zohlnhöfer D, Klein CA, Richter T, Brandl R, Murr A, et al. (2001) Gene expression profiling of human stent-induced neointima by cDNA array analysis of microscopic specimens retrieved by helix cutter atherectomy: Detection of FK506-binding protein 12 upregulation. Circulation 103: 1396-1402.
10. Malik N, Francis SE, Holt CM, Gunn J, Thomas GL, et al. (1998) Apoptosis and cell proliferation after porcine coronary angioplasty. Circulation 98: 1657-1665.
11. Komatsu R, Ueda M, Naruko T, Kojima A, Becker AE (1998) Neointimal tissue response at sites of coronary stenting in humans: macroscopic, histological, and immunohistochemical analyses. Circulation 98: 224-233.
12. Finn AV, Nakazawa G, Joner M, Kolodgie FD, Mont EK, et al. (2007) Vascular responses to drug eluting stents: importance of delayed healing. Arterioscler Thromb Vasc Biol 27: 1500-1510.
13. Suzuki T, Kopia G, Hayashi S, Bailey LR, Llanos G, et al. (2001) Stent-based delivery of sirolimus reduces neointimal formation in a porcine coronary model. Circulation 104: 1188-1193.
14. Serruys PW, Onuma Y, Garg S, Vranckx P, De Bruyne B, et al. ARTS II Investigators (2010) 5-year clinical outcomes of the ARTS II (Arterial Revascularization Therapies Study II) of the sirolimus-eluting stent in the treatment of patients with multivessel de novo coronary artery lesions. J Am Coll Cardiol 55: 1093-1101.