Hypercholesterolemia impairs vascular remodelling after porcine coronary angioplasty

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Received 15 October 2001; accepted 22 March 2002

Abstract

Objective: To assess the effect of hypercholesterolemia on neointima formation and vascular remodelling after porcine coronary angioplasty. Methods: Left anterior descending coronary angioplasty was carried out in five control and 16 age-matched hypercholesterolemic miniature pigs. Vascular remodelling was measured by intravascular ultrasound. Neointima size and composition were assessed by quantitative image analysis. Coronary smooth muscle cells (SMC) from control and diet pigs were collected 1 h after angioplasty for in vitro study of the effect of hypercholesterolemic serum on SMC migration and of macrophage-induced matrix degradation on SMC adhesion.

Results: Twenty-eight days after angioplasty, lumen increase was 0.08 ± 1.7 mm in diet and 2.7 ± 2.7 mm (P = 0.016) in control pigs. Lumen increase correlated with vascular remodelling (IEL/IEL; R² = 0.59; P < 0.001) and with the circumferential gain relative to the neointima (R² = 0.32; P < 0.01) but not with neointimal area that was similar in control and diet pigs. Circumferential gain correlated with VSMC deposition at the site of the injury (R² = 0.28; P < 0.01) that correlated with organized collagen (R² = 0.34; P < 0.01). The VSMC and collagen content of neointima in diet pigs was lower whereas the macrophage content was higher. Hypercholesterolemic serum and oxidised LDL reduced migration of VSMC from diet pigs. Macrophage-induced degradation of VSMC extracellular matrix reduced VSMC adhesion (P = 0.015). Conclusion: Hypercholesterolemia impairs vascular remodelling of balloon-treated coronary arteries. It decreases VSMC and collagen accumulation at the site of injury. Our in vitro data suggest that this decrease can be due to macrophage-induced matrix degradation and reduced VSMC adhesion and to impaired VSMC migration. Oxidised LDL mimics the inhibitory effect of hypercholesterolemic serum.

Keywords: Angioplasty; Atherosclerosis; Lipoproteins; Macrophages; Smooth muscle

1. Introduction

Restenosis after successful percutaneous transluminal coronary angioplasty (PTCA) is due about 30% to neointima formation and about 70% to constrictive remodelling [1,2]. Cellular and molecular mechanisms leading to vessel constriction remain largely unknown. Alterations in collagen metabolism, apoptotic turnover and phenotypic changes in SMC have been implied [3,4]. Inflammatory responses in the adventitia can contribute to vascular remodelling in small animal models [5].
Hypercholesterolemia has been demonstrated to facilitate vascular shrinkage after balloon injury in animal models, but lipid-lowering strategies have failed to prevent restenosis after coronary angioplasty [6,7]. In the present study a model of coronary atherosclerosis in minipigs [8] was used to study the effect of LDL hypercholesterolemia on coronary artery remodelling in response to balloon injury. We observed impaired vascular remodelling and decreased late luminal gain after angioplasty in hypercholesterolemic miniature pigs that related to decreased vascular smooth muscle cell (VSMC) and collagen accumulation at the site of mechanical injury. To better understand the impact of hypercholesterolemia on VSMC accumulation and of matrix degradation on VSMC adhesion, we have studied the effect of control and hypercholesterolemic serum on VSMC migration and proliferation and of macrophage-induced extracellular matrix (ECM) degradation on VSMC adhesion in vitro. Furthermore, we investigated whether oxidised LDL can mimic the inhibitory effects of hypercholesterolemia on VSMC migration.

2. Methods

2.1. Animal procedures

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The Ethical Committee of the Katholieke Universiteit Leuven has approved the study. Sixteen miniature pigs were placed on a 4% cholesterol diet for 6–24 weeks before PTCA. Age-matched pigs were used as controls [8]. Lipid profiles were determined in the university hospital routine lab. Levels of circulating oxidised LDL were determined as previously described [9].

2.2. Coronary artery instrumentation and assessment of vascular remodelling

Coronary instrumentations were carried out in deep propofol anaesthesia as described before [10] and intravascular ultrasound (IVUS)-loops (IVUS Visons Five-64F/X, Endosonics, Zaventem, Belgium) of the proximal LAD were recorded. Areas of external elastic lamina (EEL), internal elastic lamina (IEL) and lumen were measured. In order to oversize the coronary arteries, a balloon (balloon/vessel ratio of 1.29±0.1) was inflated at 6 atm for 30 s in the mid-LAD of diet pigs and three times for 30 s in control pigs to match injury. A pilot experiment revealed that one balloon inflation for 30 s induced IEL rupture in only one out of four control pigs but in all diet pigs. Three balloon inflations resulted in IEL rupture in five out of five control pigs. At day 28, IVUS loops were obtained and EEL, IEL and lumen areas were determined by IVUS. Lumen change (Δlumen) was calculated from recorded IVUS images as: lumen<sub>post</sub>−lumen<sub>pre</sub>. The IEL<sub>post</sub>/IEL<sub>pre</sub> ratio was calculated as a measure for vascular remodelling.

2.3. Vascular injury assessment

Arterial injury at the sites of maximal injury was assessed as described by Schwartz: 0, IEL intact; 1, rupture of IEL only; 2, media damage; and 3, EEL rupture [11].

2.4. Neointimal hyperplasia

Targeted LAD segments were sectioned at 7-μm thickness and a section every 70 μm was stained with Verhoeff-Von Gieson. A segment of 700 μm, spanning the maximal lesion, was used to assess neointima composition. Total lipid deposition in the lesions was determined by oil-red-O. Immunostaining was carried out for macrophages, oxidised LDL, SMC (α-actin and smoothelin), and collagen type I [8,12]. Blinded analysis was performed with the Quantimet 600 image analyser (Leica, Brussels, Belgium). A colour threshold mask for immunostaining was defined to detect the red or brown colour by sampling and the same threshold was applied to all samples. The lesion area with positive colour was recorded. The amount of total versus organized collagen was determined similarly by picrosirius red staining, viewed in normal versus polarized light [13,14]. A modified TUNEL protocol for the detection of apoptotic cells in complex tissues was used [15]. IEL fracture length to neointima ratio was calculated as a measure for the circumferential gain of the injured artery relative to neointima [10]. Diet-induced atherosclerosis in the absence of mechanical injury was assessed in the LCx as reference artery.

2.5. VSMC culture

One hour after PTCA, VSMC of injured coronary arteries from control and diet pigs were isolated using the explant-outgrowth method [16] and maintained in culture in Dulbecco’s modified essential medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. All experiments were carried out with cells of less than six passages.

2.6. LDL oxidation

LDL was isolated and oxidised in vitro, as previously described [17].

2.7. Proliferation and migration of VSMC

VSMC were seeded in 12- or six-well plates in DMEM-10% FBS. Sub-confluent cells were starved for 96 h in DMEM–0.2% FBS. To measure VSMC proliferation, 0.2% FBS, 10% FBS, 10% control pig serum (CPS), 10% diet pig serum (DPS) and 10 μg/ml pig oxidised LDL plus 0.5
\[\text{MDM}–0.2\% \text{FBS} \text{for} 9 \text{days}. \text{SMC} \text{were} \text{lysed} \text{in} 125 \text{mM NH}_4 \text{for} 10 \text{min} \text{at} \text{room} \text{temperature} \text{and} \text{ECM} \text{was} \text{washed} \text{five} \text{times} \text{in} \text{H}_2\text{O} \text{and} \text{kept} \text{at} 4^\circ\text{C} \text{until} \text{use}. \]

To measure ECM synthesis, subconfluent cells were maintained in DMEM–0.2% FBS for 9 days in the presence of 5 \( \mu \text{Ci/ml} \) of \( \text{L-[2,3,4,5-^3\text{H}]proline} \) (Amersham) that was renewed every 3 days [20]. Ascorbate (50 \( \mu \text{g/ml} \)) was added daily. SMC were lysed in 125 mM NH\(_4\) for 10 min at room temperature, ECM were washed five times in H\(_2\)O and lysed in PBS containing 10 mM EDTA. Radioactivity contained in lysates was quantified in a \( \beta \)-scintillation liquid counter.

2.9. VSMC adhesion

Cell adhesion was evaluated according to a previously described protocol [21] with modifications. Human monocytic THP-1 cells were seeded on plastic or on SMC ECM at 10\(^6\) cells/well and differentiated by 10\(^{-7}\) M PMA for 72 h at 37 \( ^\circ\)C. After NH\(_4\) lysis of THP-1 cells, 125 000 SMC/well were seeded for 6 h and May-Grünwald–Giemsa positive cells were counted in 10 hpf. Data were expressed as the number of adhering cells per mm\(^2\).

2.10. Extracellular matrix degradation by macrophages

Radiolabeled VSMC-ECM was obtained as described above. After NH\(_4\) lysis of VSMC, THP-1 cells were differentiated and ECM degradation was estimated as cumulatively released cpm in the supernatant over total incorporated \( ^3\text{H}\)proline.

2.11. Statistics

Groups were compared by Mann–Whitney \( U \)-test. Correlations were determined by Spearman test. Linear regression was performed using the Prism program (GraphPad). Probability values of \(<0.05\) were considered statistically significant.

3. Results

3.1. Plasma lipid levels

Table 1 demonstrates that the cholesterol diet induced a significant increase in total cholesterol, LDL-cholesterol and HDL-cholesterol but not of triglycerides within 4 weeks. Thereafter lipoprotein and lipid levels remained unchanged. Levels of circulating oxidised LDL were significantly higher in diet pigs (\( P < 0.001\); Table 1).

3.2. Acute lumen gain and vascular injury

The acute lumen gain in control and diet pigs was similar. The increase in luminal diameter was 8.7±3.7 and 5.9±5.6\%, respectively. The injury score was also similar: 2.15±0.42 and 2.00±0.72, respectively.

3.3. Vascular remodelling and lumen change

Lumen area of coronary arteries from control and diet pigs before angioplasty was very similar as shown in Fig. 1. Twenty-eight days after angioplasty, the increase in lumen area was higher in control than in diet pigs (2.6±1.2 vs. 0.8±0.42 mm\(^2\); Fig. 1). The IEL\(_{\text{post}}\)/IEL\(_{\text{pre}}\) ratio was used as a measure for remodelling. This ratio was 1.88±0.33 in control pigs and 1.48±0.32 (\( P = 0.021\)) in diet pigs, indicating reduced vascular remodelling in diet pigs.

At the end of the experiment, seven of 16 diet pigs had atherosclerotic lesions in the non-injured LCx (reference artery) that were greater than the mean±2 S.D. of control pigs (0.45±0.14 vs. 0.12±0.04 mm\(^2\); \( P < 0.001\)). Pigs with pre-existing lesions were analysed separately from the other nine diet pigs in which the intimal area in the LCx was not different (0.07±0.03 mm\(^2\)) from that in control pigs. No significant difference was noted between the two diet groups and the control group with respect to neointimal area 28 days after injury (3.42±1.22 vs. 2.96±1.51

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diet</th>
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<tr>
<td>Total cholesterol (mg/dl)</td>
<td>56±2.9</td>
<td>498±35*</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>22±1.0</td>
<td>86±7.3*</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>25±2.1</td>
<td>402±32*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>47±7.7</td>
<td>45±5.5</td>
</tr>
<tr>
<td>Oxidised LDL (mg/dl)</td>
<td>0.63±0.17</td>
<td>3.03±1.53*</td>
</tr>
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</table>

Results are expressed as mean±S.D.

\( *P < 0.001\), versus control.
Fig. 1. IVUS evaluation of vascular remodelling of balloon-treated coronary arteries. Before angioplasty (pre), lumen area of coronary arteries of control and diet pigs was very similar. The LAD proximal of the second diagonal branch was subjected to balloon injury. Balloon injury was inflicted after matching the balloon-size for a balloon/vessel ratio of 1.3. In all pigs patent LADs were observed after balloon injury. No differences in acute gain were observed. Twenty-eight days after angioplasty (post), IVUS revealed a smaller increase in lumen area of the LAD from diet pigs compared to control pigs. The IEL \_post/IEL \_pre ratio was smaller for diet pigs indicating reduced vascular remodelling.

\[ \text{IEL}_\text{post}/\text{IEL}_\text{pre} \]

vs. \(3.72\pm0.81 \text{ mm}^2\), respectively). The IEL \_post/IEL \_pre ratio was similar for diet pigs with and without diet-induced atherosclerosis in the reference artery: \(1.40\pm0.42\) and \(1.45\pm0.32\), respectively. The lumen change was also similar for pigs with and without diet-induced atherosclerosis: \(0.48\pm1.66\) and \(-0.45\pm1.63\), respectively.

3.4. Relation between VSMC at site of injury, circumferential gain, vascular remodelling and lumen change

Fig. 2 shows that lumen change correlated with the IEL \_post/IEL \_pre ratio, as a measure for remodelling, and with the IEL fracture length-to-neointima ratio, as a measure for circumferential gain of the injured artery relative to neointima. Lumen change did not correlate with neointimal area (data not shown). The circumferential gain correlated with VSMC accumulation at the site of injury that correlated with organized collagen (Fig. 2).

3.5. Hypercholesterolemia and neointimal hyperplasia

Fig. 3 shows photomicrographs demonstrating density of lipids, macrophages, smooth muscle cells and collagen in balloon-treated coronary arteries from control and diet pigs. Hypercholesterolemia increased macrophage and lipid deposition and decreased SMC and collagen deposition. Lipid staining co-localised with macrophage accumulation. In LAD of diet pigs, lipid accumulation was observed at the base of the lesion where the IEL was ruptured, but also in the periphery of the lesions co-localising with macrophage accumulation. Here, only a few \(\alpha\)-actin positive cells were observed and collagen detection revealed large gaps in the collagen web at these sites.

Fig. 4 shows mean intima, macrophage, lipid, vascular smooth muscle cell, collagen and organized collagen area in injured coronary arteries from control and diet pigs. Although mean neointimal areas in injured coronary arteries of control and diet pigs were very similar, differences in neointimal composition were observed. Neointima from diet pigs contained more macrophages, lipids and oxidised LDL (\(0.162\pm0.025 \text{ vs. } 0.027\pm0.005 \text{ mm}^2\), \(P = 0.001\)) and less SMC and (organized) collagen than control arteries (Fig. 4).

Collagen type I occupied \(0.31\pm0.06 \text{ mm}^2\) of neointima in control and \(0.12\pm0.02 \text{ mm}^2\) in diet pigs, respectively \((P<0.01)\). The proportion of \(\alpha\)-actin positive SMC that stained positively for smoothelin was not different among groups: \(11.3\pm2.1\%\) for control and \(10.9\pm2.7\%\) for diet pigs, indicating similarly differentiated VSMC.

Mean medial areas of targeted LAD segments were very
similar in control and diet pigs: 1.44±0.29 and 1.20±0.56 mm². The mean neointima/media ratio of injured segments of coronary arteries from control and diet pigs was very similar.

3.6. Relation between hypercholesterolemia and composition of neointima

Results are presented in Table 2. Lumen change was inversely related to hypercholesterolemia ($R=-0.54; P=0.005$) that positively correlated with macrophages ($R=0.63; P=0.002$), lipids ($R=0.62, P=0.002$) and oxidised LDL ($R=0.59, P=0.004$), and negatively with VSMC ($R=-0.59; P=0.005$) and organized collagen ($R=-0.38, P=0.018$) accumulation in the neointima.

3.7. Effect of hypercholesterolemia on VSMC proliferation and migration

First, the effect of in vivo exposure of VSMC to hypercholesterolemia on in vitro migration was tested. In the presence of 0.2% FBS (baseline conditions), migration of VSMC from injured arteries was similar to that of VSMC from intact arteries (Fig. 5A). Migration of VSMC from diet pigs was however significantly lower ($P<0.01$) compared to that of VSMC from control pigs in baseline conditions (Fig. 5A). Since injury had no significant effect on migratory features of VSMC, we further focused on the effect of hypercholesterolemia after angioplasty by comparing VSMC from control and diet pigs after injury. In the presence of 10% FBS, migration of VSMC from control pigs was similar to that of VSMC from diet pigs. It was threefold increased compared to baseline conditions ($P<0.05$) (Fig. 5B). Control pig serum induced a significant increase in migration of VSMC from control and diet pigs ($P<0.001, P=0.036$, respectively) compared to 0.2% FBS (Fig. 5C). In the presence of diet pig serum, migration of VSMC from control pigs was similar to that of VSMC from diet pigs. It was significantly reduced compared to baseline conditions. In contrast, it significantly decreased migration of VSMC from diet pigs ($P<0.05$; Fig. 5C).

Proliferation of VSMC derived from diet pigs was higher than that of VSMC derived from control pigs. Proliferation of VSMC from control and diet pigs in the presence of 10% FBS, 10% control and 10% diet pig serum was similar (Fig. 5D).

3.8. Effect of macrophages on extracellular matrix degradation and VSMC adhesion

THP-1 cell-induced VSMC ECM degradation increased
Fig. 3. Photomicrographs comparing accumulation of lipids, macrophages, smooth muscle cells and collagen in balloon-treated coronary arteries from control and diet pigs. In areas of macrophage accumulation in coronary arteries of diet pigs, no \( \alpha \)-actin and collagen accumulation were observed (arrow heads indicate areas that are shown at larger magnification). In the LAD from diet pigs, lipid accumulation was observed at the base of the restenotic lesion where the IEL was ruptured, but also in the periphery of the lesions co-localizing with macrophage accumulation (arrow heads). Here, only few \( \alpha \)-actin positive cells were observed and collagen staining revealed large gaps in the collagen web at these sites (arrow heads).

from 10–20\% after 24 h to 60–70\% after 5 days (Fig. 6A). Adhesion of VSMC to pre-digested ECM decreased by 55\% compared to adhesion to native ECM (Fig. 6B). VSMC adhering to native ECM were well spread and flat (Fig. 6D) with a rich and well-organized net of \( \alpha \)-actin fibres (Fig. 6F). In contrast, VSMC adhering to pre-digested ECM were skinny (Fig. 6E) with a less elaborated \( \alpha \)-actin fibre structure (Fig. 6G).

When SMC were seeded on native ECM, control and diet pig serum had no effect on ECM synthesis by VSMC. When seeded on pre-digested ECM, VSMC synthesized more ECM in the presence of FBS and control serum. In contrast, diet pig serum failed to induce ECM synthesis (Fig. 6C).

4. Discussion

In agreement with findings in man, vascular remodelling rather than neointimal hyperplasia correlated with late lumen gain after porcine coronary angioplasty. Lumen change correlated with the circumferential gain of the injured artery that correlated with organized collagen deposition that depended on VSMC accumulation at the site of injury. In aggregate, our data indicate that accumulation of VSMC and deposition of VSMC matrix play a major role in vascular remodelling. This was hampered in the injured coronary arteries from hypercholesterolemic pigs that contained less VSMC and collagen but more macrophages.

Our in vitro studies showed that hypercholesterolemia in vivo as well as in vitro reduced migration of VSMC. Moreover, we demonstrated that oxidised LDL mimicked the effect of hypercholesterolemia. In contrast, proliferation was not affected by hypercholesterolemia in vitro. VSMC from diet pig displayed a higher proliferation rate in baseline conditions. Serum from hypercholesterolemic pigs induced an increase in VSMC proliferation similar to that obtained in the presence of 10\% FBS, in accordance with previous studies [22]. Altogether our in vitro data
suggest that the inhibitory effect of hypercholesterolemia on VSMC accumulation at the site of injury may be due to impaired VSMC migration rather than VSMC proliferation. Impaired VSMC-dependent repair function is most likely due to exposure of VSMC to plasma lipoproteins after vessel wall disruption, particularly to oxidised LDL as evidenced by the decrease in VSMC migration in the presence of oxidised LDL. Hypercholesterolemia was indeed associated with an increase in circulating oxidised LDL and led to higher oxidised LDL deposition in injured arteries of hypercholesterolemic pigs.

We observed less accumulation of SMC and collagen in macrophage-rich regions in the neointima in coronary arteries of diet pigs. This is in agreement with earlier observations that macrophages secrete metalloproteinases that degrade collagen [23]. We therefore studied the effect of macrophage-induced extracellular matrix degradation on VSMC adhesion in vitro. Adhesion of VSMC to matrix digested by differentiated THP-1 cells decreased by 55\%.

Our data are in agreement with earlier findings that in vitro macrophage-induced degradation of matrix components like collagen can decrease adhesion of VSMC [24]. In vivo, we observed that neointima size was similar in diet and control pigs after angioplasty, whereas the lesion composition was different, i.e. less accumulation of SMC and collagen and more accumulation of macrophages. In aggregate, the combination of our in vivo and in vitro data suggests that hypercholesterolemia has: (i) a direct effect via oxidised LDL on VSMC accumulation and (ii) an

<table>
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<th>Covariate</th>
<th>$R$</th>
<th>$P$-value</th>
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<tbody>
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<td>Macrophages</td>
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<td>0.002</td>
</tr>
<tr>
<td>Lipids</td>
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<td>0.002</td>
</tr>
<tr>
<td>Oxidised LDL</td>
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<tr>
<td>VSMC</td>
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<td>0.005</td>
</tr>
<tr>
<td>Collagen</td>
<td>-0.38</td>
<td>0.018</td>
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The model contained the following covariates: macrophage, lipids, oxidised LDL, VSMC and collagen content.
The effect of hypercholesterolemia on VSMC migration and proliferation in vitro. Migration was evaluated using a scratch wound assay and proliferation by measuring [3H]thymidine incorporation. Migration of VSMC isolated from coronary arteries from control and diet pigs before and after injury in the presence of 0.2% FBS, *P<0.05 versus control pig (A), after injury in the presence of 10% FBS, *P<0.05 versus 0.2% FBS (B), after injury in the presence of 10% control pig serum (CPS), 10% diet pig serum (DPS) and 10 μg/ml oxidised LDL, *P<0.05 versus 0.2% FBS; *P<0.05 versus 10% CPS (C). Proliferation of VSMC isolated from coronary arteries from control and diet pigs after injury in the presence of 0.2% FBS, 10% FBS, 10% CPS, 10% DPS and 10 μg/ml pig oxidised LDL (D).

Fig. 5. Effect of hypercholesterolemia on VSMC migration and proliferation in vitro. Migration was evaluated using a scratch wound assay and proliferation by measuring [3H]thymidine incorporation. Migration of VSMC isolated from coronary arteries from control and diet pigs before and after injury in the presence of 0.2% FBS, *P<0.05 versus control pig (A), after injury in the presence of 10% FBS, *P<0.05 versus 0.2% FBS (B), after injury in the presence of 10% control pig serum (CPS), 10% diet pig serum (DPS) and 10 μg/ml oxidised LDL, *P<0.05 versus 0.2% FBS; *P<0.05 versus 10% CPS (C). Proliferation of VSMC isolated from coronary arteries from control and diet pigs after injury in the presence of 0.2% FBS, 10% FBS, 10% CPS, 10% DPS and 10 μg/ml pig oxidised LDL (D).

indirect effect via macrophages that by degrading ECM prevent SMC adhesion and thereby accumulation.

Previously we have isolated circulating oxidised LDL from the plasma of patients with cardiovascular disease and shown that its composition is very similar to that of oxidised LDL that has been isolated from atherosclerotic plaques [25]. Thus the effect of oxidised LDL present in the plasma may mimic that of oxidised LDL present in the plaque [26]. We have however to take into account that the oxidised LDL that is obtained by metal–ion induced oxidation is more extensively modified.

Positive vascular remodelling depends on ECM synthesis by VSMC to compensate the degradation of ECM by macrophages that accumulate after angioplasty [27]. We observed that macrophages co-localised with gaps in collagen where VSMC accumulated less in hypercholesterolemic pigs. In vitro, we observed that VSMC produced more matrix in the presence of control pig serum when seeded on digested matrix than when seeded on native ECM. In contrast, in the presence of hypercholesterolemic pig serum, VSMC failed to compensate ECM degradation by macrophages, in accordance with previous in vitro findings [28] and with our in vivo observations. The increased matrix production in response to macrophage-mediated matrix degradation was however impaired in the presence of hypercholesterolemic serum. An increase in matrix production could restore the ability of VSMC to migrate towards and adhere to an injured vessel wall and thereby accumulation.

Previously we have isolated circulating oxidised LDL segment in order to contribute to the repair process. Restoration of matrix did however not occur in the presence of hypercholesterolemic serum. Our in vitro data thus suggest an inhibitory effect of hypercholesterolemia on the healing capacity of VSMC by reducing their potential to produce extracellular matrix and are in agreement with the reduced VSMC accumulation in the macrophage-rich and collagen-poor areas in the neointima of diet pigs.

Production of matrix and dispersion of VSMC in the vessel wall has thus far been considered as disadvantageous and a key feature of neointima formation. Indeed will neointima, that does not stretch out circumferentially to serve as a neomedia, encroach the lumen and lead to restenosis? Our data, however, suggest that while hypercholesterolemia affects the amount of neointimal tissue only marginally, it impairs the ability of the vessel wall to utilize newly formed tissue for its expansion. This leads to an undesirable neointimal shaping in response to mechanical injury and consequent luminal encroachment due to the lack of vascular circumferential growth.

In humans, unfavourable remodelling accounts for up to 70% of clinical restenosis after coronary angioplasty. Probufol, a cholesterol-lowering agent with antioxidant
Fig. 6. Effect of macrophages on VSMC-mediated repair in vitro. (A) VSMC ECM degradation by differentiated THP-1 cells. (B) Adhesion of VSMC from injured coronary arteries from diet pigs to plastic and to native ECM was counted in the presence and absence of differentiated THP-1 cells. (C) Synthesis of ECM by VSMC on native and pre-digested ECM by THP-1 cells in the presence of 0.2% FBS, CPS and DPS. *P<0.05 versus native ECM. Adhesion of VSMC to native ECM (D, F) and to pre-digested ECM by THP-1 (E, G) stained by May-Grünwald-Giemsa (D, E) and by a monoclonal antibody to SM α-actin (F, G).
properties, decreased restenosis by improving vascular remodelling [29,30]. Cholesterol lowering therapy improves coronary remodelling in patients with CAD [31]. Cholesterol-lowering agents such as statins failed to reduce the restenosis rate 4–6 months after angioplasty [30,32,33], although several trials seemed to show a beneficial effect [30,34,35]. It is however possible that cholesterol lowering has to be started many months before angioplasty to cause a reduction in macrophage and oxidised LDL content of the arteries and have an effect on restenosis rate.

In conclusion, we showed that hypercholesterolemia impairs vascular remodelling of balloon-treated coronary arteries. It increases macrophage accumulation and decreases VSMC accumulation at the site of injury. Our in vitro data indicate that hypercholesterolemia can impair VSMC migration and that macrophage-induced matrix degradation can reduce SMC adhesion resulting in impaired vascular remodelling after balloon dilatation in diet pigs.

Acknowledgements

GT was the recipient of a research fellowship from the DFG, Bonn, Germany and is currently a member of the Interdisciplinary Centre for Clinical Research at the University of Münster. RQ was the recipient of a visiting postdoctoral fellowship from the Fonds voor Wetenschap-pelijk Onderzoek (FWO)-Vlaanderen. PV is recipient of a research fellowship of the FWO-Vlaanderen. Interuniver-sitaire Attractiepolen (P5/01/02) to PH and FWO-Vla-anderen to PH (G.0088.02) and MK (G.0080.98N) supported this work.

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