Vitamin A: a drug for prevention of restenosis/reocclusion after percutaneous coronary intervention?

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ABSTRACT

The re-establishment of adequate blood flow in a vessel with a reduced lumen due to an atherosclerotic plaque by percutaneous vascular intervention is a well established procedure. However, the long-term outcome of such interventions is negatively influenced by the development of intimal hyperplasia/restenosis. Although extensively researched, this still represents a significant clinical problem. Retinoids, i.e. natural and synthetic derivates of vitamin A, represent a potential therapeutic compound, since they have been shown to influence the vast majority of processes that ultimately lead to reocclusion of the injured vessel. Retinoids exert their effects at the transcriptional level through their nuclear receptors. Targeting multiple processes, i.e. proliferation, migration, extracellular matrix composition and cell differentiation, as well as coagulation/fibrinolysis, should increase their future role in the prevention of restenosis. The purpose of this review is to summarize the diverse effects of retinoids on pathobiological and biological processes activated at sites of vascular injury with particular emphasis on intimal hyperplasia/restenosis after endovascular interventions.

INTRODUCTION

Atherosclerosis is an inflammatory disease with a complex aetiology [1]. Clinically manifested atherosclerosis with impaired blood flow due to reduced vessel diameter is commonly treated with therapeutic endovascular interventions. Post-procedural anticoagulative treatment is needed to prevent acute thrombotic occlusion of the injured vessel [2]; however, the long-term outcome of endovascular intervention is negatively influenced by the development of intimal hyperplasia/restenosis [3]. A hallmark of this process is proliferation of SMCs (smooth muscle cells), but also includes several other processes such as migration, differentiation and matrix remodelling [4]. The treatment used presently to target intimal hyperplasia is stenting, which reduces, but does not eliminate, the frequency of restenosis [5]. Intravascular ultrasound studies performed in humans showed that in-stent restenosis was, to a large extent, caused by neointimal hyperplasia related to vessel trauma during the procedure [6]. Subsequent pathological studies confirmed that disruption of the internal elastic lamina and protrusion of stent struts into lipid-laden portions of the plaque, as well as the presence of neovascularization in the neointima, contributed to an inflammatory component of the process and was associated with increased rates of restenosis [7,8]. Systemic pharmacotherapy was proposed to tackle the problem [9], but the true revolution

Key words: atherosclerosis, intimal hyperplasia, percutaneous vascular intervention, restenosis, retinoid, vascular injury, vitamin A.

Abbreviations: DES, drug-eluting stent; ECM, extracellular matrix; KLF5, Krüppel-like zinc-finger transcription factor 5; MHC, myosin heavy chain; MMP, matrix metalloproteinase; PAI-1, plasminogen activator inhibitor-1; RA, retinoic acid; atRA, all-trans RA; RAR, RA receptor; RARE, RA-response element; SMC, smooth muscle cell; tPA, tissue plasminogen activator; VSMC, vascular SMC.

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came with the advent of DES (drug-eluting stents) coated with anti-inflammatory or antiproliferative (sirolimus and paclitaxel) compounds [10–13]. This greatly diminished the rate of in-stent restenosis (0–9 %), but created another set of problems such as increased risk of in-stent thrombosis [14–17], delayed healing and polymer hypersensitivity [15,18], and, at the present time, little is known regarding the optimal treatment of in-stent restenosis following DES implantation.

Vitamin A (retinol) and its naturally occurring biologically active derivate (atRA [all-trans RA (retinoic acid)]) are involved in multiple biological processes and implicated in active processes at the site of vessel injury, including SMC differentiation and proliferation [19], as well as possessing anticoagulant properties [20] (Figure 1).

Retinoids exert their effects via regulation of gene expression through binding to their nuclear receptors RAR (RA receptors α, β and γ) and RXR (retinoic X receptors α, β and γ) [21]. These receptors act as ligand-activated transcription factors, which regulate the expression of retinoid-responsive genes. A growing list of retinoid-regulated genes has emerged, including many genes of vascular importance [22]. Retinoids exert their cellular effects at the transcriptional level. For this to occur, active retinoid ligands are needed. Retinoid metabolism and generation of active retinoid ligands is complex, involving multiple binding proteins and metabolic enzymes [23]. The intracellular level of active retinoid ligands is not only dependent on synthesis of active retinoid ligands, but also on catabolism in which RA is degraded into polar, biologically inactive, metabolites [24]. Given the fact that retinoids are involved in many biological processes [19], it is important that its cellular level is tightly regulated. This review will describe the pleiotropic effects of vitamin A on cellular processes activated at sites of vascular injury. Our aim is to highlight the effects of vitamin A, which hopefully will serve as a basis in the development of future therapeutic approaches in the treatment of vascular restenosis.

**VITAMIN A AND MATRIX REMODELLING**

The former view that the ECM (extracellular matrix) is a purely structural component has changed. It is now recognized that the ECM has important functions in vascular homeostasis/integrity, such as regulation of migration of embedded cells. In addition, it has been shown that ECM can harbour mitogenic and chemotactative substances. Vascular cells regulate a continuous balance between ECM synthesis and degradation. Retinoids have been shown to modulate both synthesis of ECM components, as well as degrading enzymes, in VSMCs (vascular SMCs) *in vitro* and *in vivo*. Retinoid-treated SMCs had a decreased deposition of fibronectin, thrombospondin-1 and matrix Gla protein [31,32], as well as increased expression of collagen-1 and elastin [31,33]. Matrix-degrading processes are mediated by MMPs (matrix metalloproteinases), as well as the plasminogen system, mainly tPA (tissue plasminogen activator) and uPA (urokinase-type plasminogen activator). Retinoid-treated SMCs have been shown to down-regulate at least four members of the MMP family (MMP1, MMP2, MMP3 and MMP9) [31,34–36]. Furthermore, these cells have increased expression of TIMP-1 (tissue inhibitor of metalloproteinases-1), an endogenous MMP inhibitor [34]. tPA is an activator of plasminogen from which plasmin is derived. Plasmin may induce the degradation of many extracellular proteins either directly or through the activation of latent MMPs [37]. Intimal SMCs have increased expression of tPA and have been shown to

**VITAMIN A AND INTIMAL HYPERPLASIA/RESTENOSIS**

The most used animal model to study novel treatments of intimal hyperplasia is the rat balloon injury to the common carotid artery model. This model is attractive in aspects of technical easiness as well as high reproducibility. However, because of the lack of pre-existing atherosclerotic plaques at the site of injury and, hence, inflammatory activity, this method must be evaluated in different models in other species. Over the last few years, several studies have shown beneficial effects of administering retinoids in the prevention of intimal hyperplasia/restenosis after vascular interventions in several different experimental protocols and animal species (i.e. rats, rabbits and mice).

Miano et al. [25] showed, for the first time, that orally administered retinoids inhibit the formation of intimal hyperplasia after endovascular procedures in the rat model. This study was extended by Neuville et al. [26], who showed that this effect was mainly an RARα-mediated mechanism. Furthermore, they demonstrated that intimal SMCs are more susceptible to growth inhibition by retinoids than medial SMCs. A 76 % reduction in the cross-sectional area of the carotid neointima, which resulted in a 33 % increase of the luminal area, was observed. Interestingly, retinoids also seem to favour re-endothelialization, as well as improved endothelial function, as shown by Lee et al. [27]. This will ultimately lead to decreased risk of thrombosis, since an intact endothelial lining has anticoagulative properties. Retinoids are also effective in the inhibition of restenosis in vessels affected by pre-existing [28,29], studies which have been repeated with locally administered retinoids [30]. Overall, these studies show that the favourable effects of retinoids in the prevention of intimal hyperplasia/restenosis is not species- or experimental setup-dependent and offer a firm base of knowledge in future human studies.
Vitamin A: a drug for prevention of restenosis/reocclusion after percutaneous coronary intervention?

Figure 1  Summary of possible effects of retinoids on vascular processes

have higher proteolytic activity compared with medial SMCs [38]. Furthermore, atRA increases the expression of tPA through an RARE (RA-response element) within the promoter region [39]. Hence retinoids may increase the expression of ECM constituents and decrease matrix degradation through the MMPs or increase the proteolytic activity through the tPA system. However, retinoids have been shown to induce PAI-1 (plasminogen activator inhibitor-1) in VSMCs, thereby decreasing the proteolytic activity of the plasminogen activator system [40]. Thus the net effect of retinoids appears to favour ECM production with increased vascular integrity. However, the local activity and balance of proteolytic processes, i.e. MMPs and the plasminogen activator system, may rely on the experimental setup and influence the outcome of retinoid treatment. Decreased vascular integrity, seen in many vascular diseases such as atherosclerosis and restenosis, precedes many processes including migration and cell proliferation in response to vascular injury. Thus retinoids may regulate these processes through modulation of ECM composition and, hence, vascular integrity.

VITAMIN A AND SMC MIGRATION

Migration of medial SMCs towards the intima is an early hallmark in the development of restenosis after vascular interventions. Migration depends on ECM remodelling and cell–ECM adhesive properties, as well as presence of chemotactic agents. Thus ECM composition and integrity play a major part in regulating cell migration within the vessel wall. Furthermore, as mentioned above, the ECM harbours chemotactic agents which are released upon matrix degradation. In injured vessels, β1-integrin, responsible for SMC matrix adhesion, is down-regulated [41]. Decreased expression of β1-integrin precedes phenotypic modulation of SMCs and allows the cells to migrate. Retinoids have been shown to up-regulate β1-integrin [42]. This, together with, as mentioned above, increased ECM and decreased matrix degradation through inhibition of MMPs, should subsequently lead to decreased migration of SMCs. However, retinoids increase the expression of tPA, which is generally considered to be a facilitator of cell migration. Indeed, contradictory findings do exist with both decreased migration rate [31,36,43], as well as increased migration of retinoid-treated SMCs [26,44]. These discrepant findings highlight the complexity of the migratory process of SMCs, since retinoids have been shown to influence several processes that precede cell migration. The outcome appears to be related to the experimental setup, and further studies are warranted to determine the net effect in vivo.

VITAMIN A AND SMC PROLIFERATION

In restenosis, VSMCs and ECM are the main constituents which ultimately reduce luminal size. Thus SMC proliferation is believed to play a pivotal role in this process. Over the last number of years, contradictory reports on the proliferative effect of retinoids on VSMCs have emerged. An early finding showed mitogenic effects of atRA on SMCs [45], whereas others have shown no effect of retinoids on SMC proliferation [46]. However, the vast majority report growth-inhibitory effects of retinoids on VSMCs of different species from rats to humans [26,35,47,48]. Overall, it appears that retinoids stimulate quiescent SMCs in contrast with mitogen-stimulated SMCs, where growth inhibition is seen [49]. Retinoids have been shown to inhibit the proliferative effect of several mitogenic factors on SMCs such as PDGF-BB (platelet-derived growth factor-BB) [35,47], AngII (angiotensin II) [50], serum [33], serotonin [51], endothelin-1 [49] and bFGF (basic fibroblast growth factor) [52]. The mechanisms behind this inhibitory effect are still somewhat unclear and appear to involve multiple mechanisms in the mitogenic signalling pathway,
and are probably downstream of the early responses to mitogenic stimuli. This growth inhibition has been suggested to be located at cell cycle checkpoints, since retinoids have been shown to target multiple genes for cyclins and cyclin-dependent kinases in SMCs [48,49,52]. The novel KLF5 (Krüppel-like zinc-finger transcription factor 5) was identified and shown to be markedly induced in activated VSMCs [53]. KLF5 is up-regulated in the neointima within vascular lesions, and heterozygous KLF5-knockout (Klf5+/−) mice have a marked decrease in intimal and medial thickening after vascular interventions compared with wild-type animals [54]. Interestingly, Shindo et al. [54] found that RAR ligands affected KLF5 transcriptional activity by a direct physical interaction between KLF5 and ligand-activated RARs, thus indicating a putative mechanism in retinoid-mediated growth inhibition of activated VSMCs [54]. Furthermore, Sakamoto and co-workers [55] showed that outgrowth of VSMCs from human atherectomy specimens in vitro was associated with increased expression of KLF5. The studies above clearly demonstrate the inhibitory effect of retinoids on SMC proliferation. Findings have appeared that clarify some of the mechanisms behind this effect.

**VITAMIN A AND APOPTOSIS OF SMCs**

Apoptosis of SMCs may influence the formation of intimal hyperplasia and plaque evolution [56,57], and the size of the SMC population in atherosclerotic and restenotic lesions relies on the balance between cell growth and apoptosis [57]. Retinoids have been shown to induce apoptosis in some cancer cell lines [58], as well as fibroblasts, via the Fas/FasL (Fas ligand) system [59]. Similar effects have also been seen in VSMCs both in vitro [60,61] and in vivo [34]. Ou and co-workers [60] suggested that the increased apoptosis was due to increased expression of tissue transglutaminase, a protein involved in the formation of apoptotic bodies. Indeed, tissue transglutaminase inhibitors blocked the retinoid-induced apoptosis of SMCs [60]. Interestingly, intimal SMCs have been shown to be more susceptible than medial SMCs to retinoid-induced apoptosis [61]. In conclusion, retinoids inhibit proliferation and stimulate apoptosis of SMCs, preferentially of intimal origin with a suggested high proliferation rate [61], which may decrease intimal hyperplasia after vascular interventions.

**VITAMIN A AND SMC DIFFERENTIATION**

Retinoids are used clinically in the treatment of disease processes involving cell hyperproliferation and dedifferentiation, such as psoriasis and cancer [62,63]. As discussed above, a phenotypic switch from a contractile to a synthetic SMC phenotype is the central process in the response to injury to the vessel and is associated with decreased expression of differentiation markers. Retinoids have been shown to increase the expression of the SMC differentiation markers smooth muscle MHC (myosin heavy chain) [31], α-actin [28,30,31,43,64,65] and others. Although most studies show increased expression of α-actin after retinoid treatment, contradictory results do exist. Neuvile et al. [26] showed a decreased expression of α-actin in intimal SMCs and no effect on medial cells after retinoid treatment. Although the same group showed that retinoids induce the transition from the epithelioid shape to the spindle one [26], which is generally believed to be associated with increased expression of smooth muscle differentiation markers, the results on differentiation in vivo is limited. Endogenous RA signalling has been shown to co-localize with the expression of the adult smooth muscle MHC during development of the ductus arteriosus [66]. Furthermore, the level of α-actin has been shown to be reduced in SMCs from vitamin-A-deficient rats compared with controls [67]. One definition of fully differentiated SMCs is the ability of these cells to respond to contractile agonists. Wright and co-workers [68] showed that the contractility of aortic SMCs could be restored in aortic rings when incubated with atRA [68]. A more recent report from the same group [67] showed a reduced contraction of aortic rings from vitamin-A-deficient rats. A study [69] from the group that identified KLF5 as a potential target for retinoid-induced inhibition of SMC proliferation showed that Am80, a synthetic RARα agonist, inhibits serum-induced down-regulation of smooth muscle α-actin and MHC both in vitro as well as in vivo. Thus it appears that retinoids are involved in the maintenance of a contractile-competent differentiated SMC phenotype, which may, together with increased ECM integrity, limit the proliferative response of SMCs after vascular injury.

**VITAMIN A AND THE FIBRINOLYSIS/COAGULATION SYSTEM**

Homeostasis in the vessel wall includes a delicate balance between coagulation and fibrinolysis. However, pro-coagulant processes are activated at sites of vascular injury, which may lead to thrombus formation. Retinoids induce expression of tPA both in cultured SMCs [26] and in vivo [70–72], as well as in patients during retinoid treatment [73]. This induction is a direct transcriptional effect, since the presence of an RARE in the promoter of the tPA gene is observed [39]. As mentioned above, retinoids also increase the expression of PAI-1 in VSMCs, which may limit the proteolytic actions of tPA in the vessel wall [40]. However, the expression of PAI-1 in endothelial cells is not influenced by atRA [74]. In contrast with increased fibrinolytic activity, retinoids also decrease the coagulant properties of the vessel wall both in vitro [75] and in vivo [20]. Retinoids...
have been shown to inhibit tissue factor, a strong pro-coagulant factor in the vessels [20]. Furthermore, TXA₂ (thromboxane A₂), an important inducer of platelet aggregation and vasoconstriction, has been shown to be suppressed by retinoids in VSMCs [76]. Taken together, retinoids may prevent thrombosis through increased activity in fibrinolytic pathways, as well as decreased pro-thrombotic processes, which are of importance in preventing thrombus formation at sites of vascular injury.

The thrombotic activity at the site of vascular injury/stent is so profound that it requires anticoagulant treatment for thrombi to be prevented. Thus the anticoagulative and pro-fibrinolytic properties of retinoids should therefore be of benefit.

FUTURE PERSPECTIVES AND CONCLUDING REMARKS

The studies described in the present review suggest that retinoids influence a vast number of processes, all of which are important factors in the development of restenosis and thereby may represent a potential future therapeutic compound. This is supported further by the present clinical use of retinoids in proliferative disorders such as psoriasis. The limitations would be adverse side effects, which should be minimized by locally administered drugs, i.e. coated stents. Since retinoids are used clinically today in the treatment of haemopoietic malignancies and dermatological diseases, therapeutic doses, side effects and interactions with other pharmaceuticals have been defined; this is obviously an advantage when setting up future clinical trials in the treatment of restenosis. An often-neglected potential problem is the development of retinoid resistance [77]. Findings indicate that this is, at least partly, due to induction of the catalytic enzyme CYP26, belonging to the cytochrome P450 system [78,79]. One approach to reduce these limitations is the generation of synthetic retinoid agonists with high specificity to each isoform of the RAR [80] and resistant to CYP26-mediated catabolism.

An alternative to exogenous addition of active retinoid ligands is an increase of endogenous retinoid levels by blocking retinoid catabolism [81]. Such inhibitors are available and have been reported to increase endogenous levels of RA with effects mimicking those of RA [82]. Interestingly, early clinical trials of these inhibitors in the treatment of patients with psoriasis have been successful with only mild side effects [83]. Our preliminary findings show that VSMCs express CYP26, the enzyme responsible for RA degradation [84]. Blocking this enzyme results in increased cellular levels of active retinoid ligands and increased expression of RA-regulated genes, as well as decreased mitogenesis of VSMCs. This strategy would increase the local concentration of active retinoid ligands in tissues with high retinoid metabolism. Interestingly, we have shown recently [85] that synthetic intimal SMCs with high proliferative capacity have a higher retinoid metabolism compared with medial SMCs.

In summary, vascular injury and inflammation are complex pathological processes that involve many cellular events, including cell growth/differentiation and migration, as well as coagulatory and inflammatory processes. Future successful therapeutic compounds should interfere with many of these processes, rather than one specific trait as do most of the treatments currently available. Retinoids have been shown to regulate many of these disease-promoting processes, and may thereby represent a potential future candidate for prevention of intimal hyperplasia/restenosis.

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Vitamin A: a drug for prevention of restenosis/reocclusion after percutaneous coronary intervention?


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