Retinoic Acid Regulates Arterial Smooth Muscle Cell Proliferation and Phenotypic Features In Vivo and In Vitro Through an RARα-Dependent Signaling Pathway

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Abstract—We have recently shown that all-trans retinoic acid (tRA) modulates arterial smooth muscle cell (SMC) morphologic features and biochemical composition in vitro. It has been proposed that different SMC phenotypes coexist in arteries, which may be retrieved in culture: hence, a differential action of tRA on distinct SMC subsets is conceivable. We have examined the effect of tRA on SMC proliferation, migration, plasminogen activator activity, and α-smooth muscle actin expression in 2 phenotypically different rat SMC populations, cultured respectively from the normal aortic media and from the intimal thickening (IT) after endothelial injury. tRA inhibited proliferation and increased migration and tissue-type plasminogen activator activity in both SMC populations, but decreased α-smooth muscle actin only in SMC cultured from the IT. The action of tRA is mediated by 2 families of nuclear receptors, RAR and RXR, each containing 3 isoforms, α, β, and γ. RAR and RAR-α agonists, but not RXR agonists, inhibited SMC proliferation in both cell populations and α-smooth muscle actin expression only in IT SMC. When administered intraperitoneally to balloon-injured rats, tRA and RAR-α agonists reduced the intimal hyperplasia in the carotid artery. Our results show that tRA and synthetic retinoids can affect the proliferation, migration, and differentiation of SMC in vitro. Furthermore, retinoids are able to reduce the IT induced by endothelial injury in vivo. (Arterioscler Thromb Vasc Biol. 1999;19:1430-1436.)

Key Words: smooth muscle cell heterogeneity ■ smooth muscle cell motility ■ smooth muscle cell differentiation ■ intimal thickening

Smooth muscle cell (SMC) differentiation, migration, and proliferation are key factors in the development of atherosclerosis and restenosis after angioplasty. These phenomena involve an SMC phenotypic modulation characterized by modification of gene expression. It has been suggested that SMC subsets present in the normal media are particularly prone to undergo phenotypic modulation, and the concept of SMC heterogeneity is gaining wider acceptance (for review see Reference 4). For instance, SMC populations cultured from the rat aortic media and from the intima 15 days after endothelial injury are characterized by a spindle-shaped and an epithelioid morphology, respectively (for discussion of this point see Reference 5). These 2 phenotypes are also obtained when SMC are cloned from the aortic media or the intima, albeit in different proportions.

The regulation of SMC phenotype is thought to be exerted by cytokines, growth factors, and agents regulating differentiation. Within the last category, some agents are known to govern SMC differentiation during embryogenesis, and there is a growing body of evidence suggesting that the genetic programs used during embryogenesis may act also during arterial disease processes.

Vitamin A plays a crucial role in the regulation of cell growth and differentiation, and its active form, retinoic acid, is involved in signal transduction pathways regulating embryonic development. These effects are mediated by 2 families of nuclear receptors that are ligand-dependent transcription factors, the RARs and the RXRs, each with 3 isoforms, α, β, and γ. Recently, we have observed that a protein involved in the retinoid pathway, cellular retinol-binding protein-1 (CRBP-1), is expressed selectively in the epithelioid SMC populations and clones.

The aim of the present study was to explore the possibility that retinoids influence differentially the features of distinct SMC phenotypes. We show that all-trans retinoic acid modulates proliferation, migration, plasminogen activator (PA) activity, and α-smooth muscle (α-SM) actin expression in SMC. By using a series of RAR and RXR agonists, we present evidence that these effects are mediated through an RARα-dependent signaling pathway, the stimulation of...
which inhibits the development of intimal thickening (IT) after arterial injury in vivo.

Methods

In Vivo Experimental Procedures

Adult male Wistar and Sprague-Dawley (body weight 300 to 400 g) rats were used for the experiments. Anesthesia was induced with enflurane (Ethrane, Abbott Laboratories) and then maintained with an intraperitoneal injection of pentobarbital sodium (Nembutal, Abbott Laboratories). Angioplasty of rat carotid artery and thoracic aorta was achieved by removal of the endothelium using an inflated embolectomy catheter according to Baumgartner and Studer. Animals were sacrificed with an overdose of pentobarbital sodium in 4% buffered formaldehyde, and embedded in OCT. The thoracic aortas were excised for cell isolation. Procedures involving animals were approved by the Ethical Committee of Geneva Medical Faculty and the Swedish Animal Ethics Committee.

In Vivo Drug Treatments

All-trans retinoic acid (tRA, Sigma Chemical Co) and CD336 (CIRD Galderma) were dissolved in 70% Intralipid (200 mg/mL; Pharmacia), 25% saline, and 5% ethanol, and injected intraperitoneally at a dose of 0.5 mg/kg immediately after rat carotid deendothelialization and once a day thereafter for the following 14 days. The controls were injected with the identical volume of 70% Intralipid, 25% saline, and 5% ethanol. Body weight was measured every day. Vessels were sectioned and stained with hematoxylin and eosin. The media and IT area were tracked and digitized by image analysis.

Cells and Culture Conditions

The aortic media from normal rats and the IT from injured rats were cultured in the same medium. Cells were used between the 5th and the 10th passages.

In Vitro Drug Treatments

All-trans retinoic acid, Ro13-7410 (TTNPB, RAR agonist), Ro44-4753 (RARγ agonist), Ro47-5944 and Ro48-2250 (RXR agonists), Ro25-7386 (RXRα agonist), Ro61-8431 (RARα agonist), Ro41-5253 (RAKo agonist; F. Hoffmann-LaRoche Ltd), Am80 and Am580 (RARα agonists; F. Hoffmann-LaRoche Ltd and Sumitomo Pharmaceuticals Co, Ltd), BMS 753 (RARα agonist), and BMS 961 (RARγ agonist; Bristol-Myers Squibb, kindly provided by Prof. P. Chambon, IGBMC) were dissolved in DMSO at 10⁻⁶ mol/L, then diluted in DMEM and added to the cells at 10⁻⁵ mol/L final concentration. SMCs were plated at a density of 5×10⁴ cells/cm², and retinoids were added every 2 days when medium was changed. Cells treated with the same concentrations of DMSO were used as control. Cells were counted and harvested after 7 days.

RNA Extraction and Northern Blot Hybridization

Cultured cells were scraped from tissue culture dishes using a rubber policeman in 0.8 mL of Tri reagent (Sigma) per dish. Total RNA extraction and purification were performed according to the protocol of the manufacturer. Ten micrograms of total RNA was denatured with glyoxal, separated by electrophoresis on 1% agarose gel, and blotted on hybrid-N filters (Amersham Corp). After UV fixation, filters were stained with 0.04% methylene blue in 0.5 mol/L sodium acetate to verify correct loading and transfer. Prehybridization and hybridization were performed for 3 and 16 hours, respectively, at 55°C in 5× SSC, 5× Denhardt solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA), 0.01% SDS, and 400 μg/mL salmon sperm DNA. After hybridization, filters were washed twice for 15 minutes at 55°C in 2× SSC, 0.1% SDS. They were then exposed to Kodak X-Omat SO-282 film (Eastman Kodak) at −70°C for 20 hours. Hybridizations were performed with a random priming-labeled GAPDH probe and an α-SM actin probe (prepared in our laboratory) corresponding to 120 bp of the 3′ untranslated region of the α-SM actin gene.

Cell Migration

For evaluation of cell migration, SMCs were plated at a concentration of 5×10³ cells/cm² in DMEM in the presence of 10% FCS. Subconfluent cultures were treated for 2 days with tRA and Am80 at 10⁻⁶ mol/L final concentration or with 10% FCS plus DMSO as control, and then scratched with a silicone rubber to obtain an 80-mm wide in vitro wound. Four fields were randomly selected, and photomicrographs were taken. 5-Bromo-2′-deoxyuridine (BrDU, Fluka) and 5-fluoro-2′-deoxyuridine (FdU, Fluka) were added at 25 and 2 μmol/L, respectively, for 24 hours. Then cells were fixed in methanol, and the extent of incorporation was determined by using a monoclonal antibody for BrDU (Dako). Nuclei were stained with propidium iodide (Sigma). Photomicrographs of the same fields were taken after 24 hours, and the proportion of migrating cells stained by BrDU antibody was evaluated by image analysis using the KS400 system (Kontron Elektronik).

Zymographic and Reverse Zymographic Assays

Cells were seeded into 35-mm tissue culture dishes at 10⁵ cells/dish in DMEM plus 10% FCS and grown to subconfluence. Twenty-four hours after the last medium change, culture dishes were washed twice with serum-free DMEM, and tRA was then added for 15 hours at 10⁻⁶, 10⁻⁷, or 10⁻⁸ mol/L in 1.5 mL serum-free DMEM containing Trasylol (200 KIU/mL; Bayer). For zymographic and reverse zymographic assays, culture media and cell lysates were analyzed as previously described. For reverse zymographic assays, samples were preincubated with 0.5% SDS and 0.5% β-mercaptoethanol at 1 hour at 37°C to neutralize PA activity. Twenty-microliter aliquots were subjected to SDS-PAGE and zymographic analysis using a casein- and plasminogen-containing substrate gel. Human urokinase (0.05 U/mL; Serono) was added to substrate gels for reverse zymographic analysis. Zymograms and reverse zymograms were photographed under dark-field illumination. In each experiment, cell numbers were determined in a duplicate series of dishes incubated in parallel; samples were then analyzed on the basis of cell number equivalents.

Statistical Analysis

Data are given as mean±SEM. A Student’s t test for unpaired samples was used for statistical analysis. Significant differences were accepted for P<0.05.
Results

Effect of tRA Treatment on IT and Medial SMC

We examined the effect of tRA on SMCs cultured from normal rat aortic media and IT that are respectively spindle-shaped and epithelioid. The growth inhibitory or stimulatory activity of tRA was analyzed by DNA synthesis measurements. tRA, ranging from 0.125 to 2.0 μmol/L, induced a dose-dependent inhibition of serum-stimulated [3H]thymidine uptake in IT cells (5% to 40%) but did not affect medial SMCs at any dose tested (Figure 1A). However, after a 7-day treatment, SMC proliferation was inhibited by tRA to the same extent in medial SMCs (62.50±3.51% of control values) and in IT SMCs (62.75±1.25%) (Figure 1B). These data indicate that IT SMCs are more sensitive to tRA treatment than medial cells at early times, but that after 7 days, selectivity is lost.

We also observed an effect of tRA on SMC migration. Medial and IT SMCs were cultured until subconfluence, and were treated for 2 days with tRA at 10⁻⁶ mol/L before wounding. After 24 hours, numerous SMCs invaded the empty space in both populations. SMC migration was increased by tRA treatment in both IT (209.88±11.31%) and medial cells (202.78±6.53%) compared with control cultures (Figure 2A). Despite the fact that, in absolute terms, IT SMCs migrate more actively than medial cells, 6 no significant difference in the percentage level of induction was observed between the 2 cell populations. To assess the effect of tRA on cell proliferation in this experiment, we have analyzed the proportion of migrating cells that had incorporated BrdU. The data represent the mean and SD of at least 3 separate experiments for each condition. tRA induced a dose-dependent inhibition of DNA synthesis in IT cells but did not affect medial cells at any dose tested. B, Effect of tRA on IT and medial SMC proliferation. Cultured SMCs were treated with 10⁻⁶ mol/L tRA and were counted after 7 days. After 7 days, tRA inhibited cell proliferation in both cell types.

The effect of tRA on the proteolytic activity of IT and medial SMCs caused by PA was investigated by zymographic and reverse zymographic assays. Tissue-type PA (tPA) and urokinase PA (uPA) activities were present in the culture supernatants of both SMC populations as previously reported.20 After tRA treatment, tPA increased and uPA decreased in a dose-dependent manner in both IT and medial SMCs (Figure 3, top), whereas plasminogen activator inhibitor-1 (PAI-1) was not significantly affected (Figure 3, bottom).

We studied the effect of tRA on α-SM actin expression by means of Western blot analysis. In medial SMCs a 7-day tRA treatment did not affect α-SM actin expression (107.12±1.39%) whereas it induced a significant decrease in IT SMC (63.66±0.46%) (Figures 4 and 5). This effect was not observed at the mRNA level after a 7-day treatment with retinol or tRA, or during a time course of 48 hours (data not shown).

Effects of RAR and RXR agonists on IT and Medial SMCs

tRA effects are mediated by 2 families of nuclear receptors, the RARs and the RXRs. To identify the receptors responsible for the action of tRA on SMCs, we examined the effects of synthetic retinoid derivatives, an RAR general agonist (Ro13-7410/TTNPB) and 2 RXR general agonists (Ro47-5944 and Ro48-2250). These analogs induced growth inhibition of both IT and medial SMCs, whereas α-SM actin content was reduced to the same level as tRA-treated cells (Figures 4 and 5). This effect was not observed at the mRNA level after a 7-day treatment with retinol or tRA, or during a time course of 48 hours (data not shown).

Each receptor subfamily contains 3 members: RAR-α, RAR-β, and RAR-γ; and RXR-α, RXR-β, and RXR-γ.

![Figure 1](http://atvb.ahajournals.org/)

Figure 1. A, Dose-dependent inhibitory effect of tRA on DNA synthesis. After a 24-hour tRA treatment at doses ranging from 0.125 to 2.0×10⁻⁶ mol/L, the rate of DNA synthesis was measured by a 24-hour pulse of [3H]thymidine. The data represent the mean and SD of at least 3 separate experiments for each condition. tRA induced a dose-dependent inhibition of DNA synthesis in IT cells but did not affect medial cells at any dose tested. B, Effect of tRA on IT and medial SMC proliferation. Cultured SMCs were treated with 10⁻⁶ mol/L tRA and were counted after 7 days. After 7 days, tRA inhibited cell proliferation in both cell types.

![Figure 2](http://atvb.ahajournals.org/)

Figure 2. A, Effect of tRA and Am80 on cell migration in both cell types. B, BrdU incorporation in control cultures
Therefore, we used different derivatives to examine whether one of these receptor isotypes could account for the effect of tRA on SMC proliferation and \( \alpha \)-SM actin expression. Am80 (Ro40-6055) is an RAR-\( \alpha \)-selective agonist that inhibited SMC proliferation in medial (54.22 \( \pm \) 1.16\%) and IT (54.16 \( \pm \) 1.06\%) SMC populations (Figure 6), induced SMC migration in medial (240 \( \pm \) 12.22\%) and IT (172.33 \( \pm \) 7.38\%) SMC populations (Figure 2A) without affecting significantly BrdU incorporation in medial (97.79 \( \pm \) 4.38\%) and IT (99.78 \( \pm \) 3.30\%) cells (Figure 2B), and lowered \( \alpha \)-SM actin only in IT (62.43 \( \pm \) 2.08\%) cells (Figures 4 and 5) as tRA and RAR agonists. The same results were obtained with 2 other RAR-\( \alpha \) agonists, Am580 and BMS 753, whereas RAR-\( \gamma \) agonists (Ro44-4753 and BMS 961) had no effect (data not shown). Moreover, Ro41-5253, an RAR-\( \alpha \)-specific antagonist, abolished the effect of both RAR-\( \alpha \) agonists on \( \alpha \)-SM actin protein expression (Figures 4 and 5) and to a lesser extent on SMC proliferation (Figure 6). As control, we showed that CRBP-1 induction by Am80 in medial SMCs was abolished by the addition of the antagonist (Figure 4B).

Retinoic Acid and RAR-\( \alpha \) Agonist Inhibit Intimal Thickening After Balloon Injury in Rat Carotid Arteries

We examined the effect of retinoids on the arterial wall in vivo using the classic endothelial injury model. tRA and CD336 (both at 0.5 mg/kg body weight) were injected intraperitoneally daily for 14 days subsequent to balloon catheter injury of the carotid artery. In vehicle-treated control rats, extensive IT (0.114 \( \pm \) 0.014 mm\(^2\)) was observed in all injured vessels 14 days after injury (Figure 3). In rats treated with tRA and CD336, however, the extent of intimal thickening was significantly reduced (0.047 \( \pm \) 0.009 mm\(^2\)). These data suggest that retinoids may have potential therapeutic applications in the treatment of atherosclerosis.

![Figure 3](http://atvb.ahajournals.org/)

**Figure 3.** Zymographic and reverse zymographic assays of culture supernatants prepared from IT and medial SMCs treated with 10\(^{-9}\), 10\(^{-7}\), and 10\(^{-6}\) mol/L tRA. Results showed that tRA increased tPA and decreased uPA activities (top), but did not affect significantly PAI-1 (bottom) in both cell types.

![Figure 4](http://atvb.ahajournals.org/)

**Figure 4.** Representative Western blots showing \( \alpha \)-SM actin expression in IT (A) and medial SMCs (B) treated with tRA and an RAR-\( \alpha \) agonist and antagonist. Am80 decreased \( \alpha \)-SM actin expression selectively in IT SMCs. As control we showed that Am80 increased CRBP-1 expression but did not affect \( \alpha \)-SM actin expression in medial SMCs (B). Ro41-5253 antagonized the action of Am80 both on \( \alpha \)-SM actin in IT cells and on CRBP-1 in medial cells.

![Figure 5](http://atvb.ahajournals.org/)

**Figure 5.** Effect of RAR-\( \alpha \) agonist on \( \alpha \)-SM actin expression in IT and medial SMCs. Cultured SMCs were treated with 10\(^{-8}\) mol/L tRA, Am80 (RAR-\( \alpha \) agonist), Ro41-5253 (RAR-\( \alpha \) antagonist), and simultaneously with both Am80 and Ro41-5253. \( \alpha \)-SM actin expression was evaluated by means of Western blot analysis. Am80 selectively inhibited \( \alpha \)-SM actin expression in IT SMCs. This effect was fully inhibited by the addition of the antagonist.

![Figure 6](http://atvb.ahajournals.org/)

**Figure 6.** Effect of RAR-\( \alpha \) agonist on IT and medial SMC proliferation. Cultured SMCs were treated with 10\(^{-6}\) mol/L tRA, Am80 (RAR-\( \alpha \) agonist), Ro41-5253 (RAR-\( \alpha \) antagonist), and simultaneously with both Am80 and Ro41-5253. Cells were counted after 7 days. Am80 inhibited cell proliferation in both cell types to the same extent as tRA. This effect was partially inhibited by the addition of the antagonist.
after injury (Figure 7). In contrast, tRA-treated rats showed a 76% (0.028±0.018 mm²) reduction in the cross-sectional area of the carotid neointima (Figure 7). The media itself was not affected by tRA (0.061±0.004 mm² versus 0.070±0.005 mm² for the controls). As a consequence of the reduction of the IT, the diameter of the lumen was increased by 33% in the tRA-treated rats (0.677±0.0228 mm versus 0.508±0.0328 mm for the controls).

The RAR-α agonist, CD336, also reduced neointima formation after balloon catheter injury (0.035±0.021 mm²) but the effects were less pronounced than those observed using tRA (Figure 7). Inhibition of IT by tRA and RAR-α agonists was obtained also after balloon-induced lesion of the rat aorta (data not shown).

There was no difference in body weight between animals treated with retinoids and vehicle, and no signs of toxicity were observed in the animals.

**Figure 7.** Effect of tRA and CD336 on the formation of neointima in balloon-injured rat carotid arteries. Representative cross-sections (×200) of the injured carotid arteries from rats treated for 14 days with vehicle (A, n=7), tRA (B, n=6), and CD336 (C, n=6) are shown. D, Summary of the effects of tRA and CD336 on neointima formation in rat carotid arteries. Media and IT area was measured by morphometric analysis. tRA did not affect the area of the media and decreased area of the intima.

**Discussion**

We have previously reported that CRBP-1, a protein involved in retinoid metabolism, is selectively expressed by a subpopulation of rat SMCs prone to migrate, proliferate, and give rise to intimal hyperplasia. Moreover, we have shown that tRA induces morphological changes in this SMC subset. Retinoids are known for their ability to inhibit cell growth and promote differentiation of many cell types. They are used for the treatment of several disease processes involving cell hyperproliferation or dedifferentiation such as psoriasis and cancer.

The action of retinoids on SMC growth has been the subject of contradictory reports indicating that tRA either increases or decreases SMC proliferation. It is now considered that tRA activates mitogenesis in quiescent SMCs and decreases growth-stimulated SMC proliferation, suggesting that more than a single mechanism accounts for the growth-regulatory activity of tRA. In this study, we demonstrate that, in serum-stimulated conditions, tRA inhibits both medial and IT SMC proliferation in vitro and that this effect is more rapid on IT SMCs. The difference in retinoid sensitivity can be explained by a difference in growth rate of the 2 populations, because IT SMCs grow more rapidly than medial cells. Indeed, when both populations reached confluence, the tRA-mediated growth-inhibition was identical. IT SMCs may also be more sensitive to tRA because of their ability to metabolize retinoids as indicated by the presence of CRBP-1. A positive correlation exists between the level of CRBP-1 and retinoid responsiveness, and cells that metabolize retinoic acid generally are growth-inhibited by tRA.

We examined the effect of retinoids on SMC migration in vitro and found that tRA stimulated medial and IT SMC migration to the same extent. This effect is not caused by increase proliferation, because in serum-stimulated conditions, tRA inhibits SMC growth. Moreover, BrdU incorporation indicates that proliferation is not significantly modified by tRA treatment during the migration test.

Migration, which is essential for neointima formation, requires degradation of extracellular matrix components. Enzymes involved in matrix degradation belong either to the matrix metalloproteinases (MMPs) or to the PA system. Many of them are tRA-regulated genes such as stromelysin, collagenase, or tPA. MMPs are negatively regulated by tRA, and this inhibition is accompanied by a tRA-mediated activation of genes encoding extracellular matrix components such as fibronectin, laminin, and collagen IV. In SMCs, the production of pro-MMP-1 is downregulated by tRA. These observations suggest that tRA may reduce SMC migration. However we observed that this process was stimulated by tRA. On the basis of this finding, we investigated whether tRA modulates the PA system in both SMC populations. These enzymes, mainly uPA and tPA, are activators of the plasminogen from which plasmin is derived. In turn, plasmin may induce the degradation of many extracellular proteins either directly or through the activation of latent MMPs. We have recently reported that tPA is the main PA involved in the proteolytic activities of both SMC populations and that IT SMCs exhibit a higher proteolytic activity, mainly because of increased expression of tPA. Similar results were obtained when IT and medial SMCs were treated by basic fibroblast growth factor and platelet-derived growth factor, 2 cytokines that induce SMC migration. It is noteworthy that a stimulation of tPA expression by tRA has been described in endothelial cells. Finally, an activation of tPA expression has been reported, during SMC migration from the media to the intima, by cells located in the luminal layer, where CRBP-1, and consequently retinoid activity, is maximal.

Taken together, these observations suggest that epithelioid IT SMCs expressing CRBP-1 are more prone than spindle-shaped medial SMCs to respond to retinoids by decreasing their growth rate and by increasing their migrating activity through tPA activation. It also is tempting to speculate that retinoids may have distinct roles depending on the retinoid sensitivity of SMCs: they might decrease migrating activities mediated by MMPs and enhance extracellular matrix deposition, or they might increase migrating activities mediated by the PA system.
We further investigated the effect of tRA on expression of α-SM actin, one of the SMC differentiation markers. We found that α-SM actin expression was selectively inhibited by tRA in IT SMCs, whereas it remained unchanged in medial cells. The observation that tRA decreases α-SM actin expression is surprising because tRA is considered as a potential inducer of SMC differentiation, at least during development.40 It has also been shown that P19 embryonal carcinoma cells and dedifferentiated rat aortic SMCs (A7r5) exposed to tRA express α-SM actin, suggesting tRA-induced SMC differentiation.41–44 The apparent discrepancy between these findings and our data may have at least 2 explanations. First, the level of differentiation of cultured SMCs may influence their sensitivity to tRA. It is conceivable that tRA exerts differentiating effects only on poorly differentiated SMCs (eg, containing low levels of α-SM actin); this is not the case for medial and IT SMCs in culture that express α-SM actin at comparably relatively high levels. Second, tRA may increase or decrease α-SM actin expression acting at different metabolic steps. Many genes, such as CRBP-1, are regulated by tRA at the transcriptional level through interactions between RA receptors and an RA response element (RARE) located in the promoter region.45,46 This is not the case for α-SM actin, and the tRA-mediated downregulation that we observed in differentiated SMCs is certainly posttranscriptional, because no variation of α-SM actin mRNA was observed in tRA-treated SMCs. This suggests that a hitherto unknown mediator of retinoid action acts at the α-SM actin translational level.

We also tried to shed light on the receptor(s) involved in retinoid signaling events. It has been previously reported that 5 of the 6 nuclear receptors are expressed in rat SMCs in vitro and in vivo.25 We used agonists specific for the different RA receptors and found that RAR-α agonists modulate SMC growth, SMC migration, and α-SM actin expression in IT cells similarly to tRA. In addition, the RXR-selective retinoids were unable to elicit these events. Moreover, the action of tRA, at least on SMC proliferation and α-SM actin expression, was inhibited by the RAR-α antagonist Ro41-5253. This compound competes with tRA for binding to RAR-α.47 Taken together, these results provide evidence for the involvement of RAR-α in the transduction of the retinoid signal in SMC. This pathway may also apply to the tRA-mediated SMC differentiation of embryonal carcinoma cells, because a subclone of P19 cells (RAC65) that fails to differentiate carries a mutation in the RAR-α gene.48,49 We did not investigate the action of RAR-α agonists on the PA system, but it is of particular interest to note that tRA-induced tPA gene expression in endothelial cells is specifically mediated by RAR-α.50

Finally, we investigated the action of tRA and CD336, an RAR-α agonist, on the formation of IT after endothelial lesion in rat carotid arteries. We observed that both tRA and RAR-α agonist reduced the IT and increase the size of the lumen diameter without affecting the media. Our results confirm the observations of Miano et al.,51 published while this manuscript was under review process. In addition, we show here that RAR-α is responsible for tRA actions. Thus, our results indicate that tRA and selective retinoids are capable of blocking intima formation. Moreover, the effect of tRA on IT SMCs, without interfering with the physiological properties of medial SMCs, suggests that tRA action is exerted preferentially on this SMC subset. Therefore, identification of the target genes of tRA in IT SMCs may help to define the mechanisms underlying the sensitivity of IT cells to tRA. This knowledge may be useful for the design and evaluation of drugs capable of inhibiting intima SMC replication in vivo.

The finding that the antiproliferative role of tRA is efficient both in vitro and in vivo suggests a potential therapeutic use of tRA and analogs during hyperproliferative vascular disease. Clinical applications of tRA have had some success in the treatment of human diseases such as cancer, psoriasis, and leukemia, but its therapeutic use has been precluded by serious adverse effects such as skin or liver toxicity and teratogenesis.52 Moreover, the biological efficacy of tRA is greatly impaired by a hypercatabolism of the drug accompanied by rapid development of resistance.53 The development of new retinoic acid derivatives targeted against RAR-α exhibiting higher activity and lower toxicity than tRA could be one of the promising strategies for therapeutic intervention in arterial disease.

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