Involvement of Aldose Reductase in Vascular Smooth Muscle Cell Growth and Lesion Formation After Arterial Injury

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Abstract—Abnormal proliferation of vascular smooth muscle cells (VSMCs) is an important feature of atherosclerosis, restenosis, and hypertension. Although multiple mediators of VSMC growth have been identified, few effective pharmacological tools have been developed to limit such growth. Recent evidence indicating an important role for oxidative stress in cell growth led us to investigate the potential role of aldose reductase (AR) in the proliferation of VSMCs. Because AR catalyzes the reduction of mitogenic aldehydes derived from lipid peroxidation, we hypothesized that it might be a potential regulator of redox changes that accompany VSMC growth. Herein we report several lines of evidence suggesting that AR facilitates/mediates VSMC growth. Stimulation of human aortic SMCs in culture with mitogenic concentrations of serum, thrombin, basic fibroblast growth factor, and the lipid peroxidation product 4-hydroxy-trans-2-nonenal (HNE) led to a 2- to 4-fold increase in the steady-state levels of AR mRNA, a 4- to 7-fold increase in AR protein, and a 2- to 3-fold increase in its catalytic activity. Inhibition of the enzyme by sorbinil or tolrestat diminished mitogen-induced DNA synthesis and cell proliferation. In parallel experiments, the extent of reduction of the glutathione conjugate of HNE to glutathionyl-1,4-dihydroxynonene in HNE-exposed VSMCs was decreased by serum starvation or sorbinil. Immunohistochemical staining of cross sections from balloon-injured rat carotid arteries showed increased expression of AR protein associated with the neointima. The media of injured or uninjured arteries demonstrated no significant staining. Compared with untreated animals, rats fed sorbinil (40 mg · kg⁻¹ · d⁻¹) displayed a 51% and a 58% reduction in the ratio of neointima to the media at 10 and 21 days, respectively, after balloon injury. Taken together, these findings suggest that AR is upregulated during growth and that this upregulation facilitates growth by enhancing the metabolism of secondary products of reactive oxygen species. (Arterioscler Thromb Vasc Biol. 2000;20:1745-1752.)

Key Words: vascular smooth muscle ■ lipid peroxidation ■ restenosis ■ growth factors ■ aldose reductase

Proliferation of vascular smooth muscle cells (VSMCs) is one of the key features of atherogenesis, restenosis, and hypertension. It is preceded by endothelial dysfunction due to cardiovascular risk factors or mechanical injury, resulting in the expression of several growth factors and cytokines that exert mitogenic effects on VSMCs. Recent evidence suggests that reactive oxygen species (ROS) are essential mediators of cell signaling initiated by growth factors and cytokines. Stimulation of VSMCs by growth factors such as platelet-derived growth factor, fibroblast growth factor (FGF), and thrombin enhances ROS generation, and cell growth in response to these mitogens is inhibited by antioxidant interventions. Thus, oxidative stress, which represents a consequence and a cause of endothelial dysfunction, appears to be involved in mediating and sustaining abnormal VSMC growth during atherosclerosis and restenosis. However, the mechanisms by which ROS mediate cell growth remain unclear.

The cellular reactions of ROS are complex and involve several intermediates and end products with variable bioactivity and toxicity. Current evidence indicates that some of the downstream effects of ROS are mediated in part by products of lipid peroxidation, such as the α,β-unsaturated aldehydes. These aldehydes are the major end products generated by the oxidation of ω-6 polyunsaturated fatty acids, eg, linolenic, linoleic, arachidonic, and docosahexaenoic acids. Owing to their high electrophilicity derived from α,β conjugation, these aldehydes react avidly with cellular glutathione and form covalent adducts with nucleophilic side chains of cellular proteins. Antibodies against protein-
aldehyde adducts "stain" VSMCs proliferating in vivo,10 and in culture, low concentrations of 4-hydroxy-trans-2-nonenal (HNE) stimulate proliferation of VSMCs,11 indicating that the biochemical pathways leading to the generation and metabolism of these products may be important regulators of cell growth. Although the specific pathways regulating the detoxification of these aldehydes in VSMCs remain unknown, it has been suggested that the aldehyde reductase (AR)–catalyzed reduction is an important route of HNE metabolism in cardiovascular tissues,12 including VSMCs.13,14

The enzyme AR is a member of the aldo-keto reductase superfamily, which includes carbonyl-metabolizing enzymes involved in glucose metabolism, prostaglandin and steroid biosynthesis, and aldehyde detoxification.15,16 It is currently believed that AR represents the first and rate-limiting step of the polyol pathway. Because of its ability to generate high concentrations of osmotically active sorbitol from glucose, AR has been suggested to be responsible for the tissue injury associated with prolonged hyperglycemia. In apparent support of this view, it has been reported that AR inhibitors delay or prevent hyperglycemia-associated tissue injury.15,17 However, recent studies show that in contrast to glucose, AR is a more efficient catalyst for the reduction of medium-chain aldehydes generated during lipid peroxidation, indicating its participation in the detoxification of lipid peroxidation products.18,19 Interestingly, stimulation of NIH 3T3 cells with FGF results in a marked increase in a delayed-early gene product (FR-1) that displays structural20 and kinetic21 properties similar to those of AR. Moreover, during hepatocarcinogenesis, AR is one of the most prominent tumor-associated antigens,22 indicating a growth-related function of this enzyme. On the basis of these observations, we examined whether AR is involved in VSMC growth.

Methods

Unless stated otherwise, all chemicals were obtained from Sigma Chemical Co. Basic FGF (bFGF) was from Collaborative Biochemical. The HNE was purchased from Cayman Chemical Co. [methyl-3H]thymidine was obtained from DuPont NEN; [32P]dATP and [γ-32P]ATP were from Amersham Co. The 1-kb human AR cDNA was purchased from the American Type Culture Collection, Manassas, Va. Peroxidase-labeled goat anti-rabbit antibody was purchased from the American Type Culture Collection, Manassas, Va. Enzyme-linked immunosorbent assays (ELISAs) were made quiescent by incubation for 48 hours in Dulbecco’s modified Eagle’s medium containing 0.1% fetal calf serum. For measuring DNA synthesis and cell proliferation, the cells were grown to 50% confluence. VSMCs were used at passages 4 to 9 because no difference in responsiveness was noted within this range.

Northern and Western Blot Analyses

Quiescent VSMCs were treated with mitogens (10% serum, 20 ng/mL bFGF, 2 U/mL α-thrombin, or 2.5 μmol/L HNE) for the indicated times. The VSMCs cultured with 0.1% serum for the same duration served as controls. Isolation, fractionation, and hybridization of RNA were performed as described previously.10 The 1-kb human AR cDNA probe was labeled with [3H]dATP by random priming and used to hybridize nicotinucleosome filters. The filters were also hybridized with an 18S rRNA probe to correct for loading differences. Western blots were developed with a polyclonal anti-AR antibody.

DNA Synthesis and Cell Proliferation

Growth-arrested VSMCs were treated with serum, 2 U/mL thrombin, or 2.5 μmol/L HNE in the presence or absence of the AR inhibitors, which were added 30 minutes before stimulation with the mitogens. For radiolabeling, 1 μCi/mL of [methyl-3H]thymidine was added 24 hours before the end of the incubation period, and DNA synthesis was measured as trichloroacetic acid–precipitable material as described previously.10 In brief, cells were resuspended in 20% trichloroacetic acid, placed on ice for 15 minutes, and filtered by passage through glass fiber filters (Whatman International Ltd). The filters were washed with cold 5% trichloroacetic acid and 80% ethanol and dried. [3H]thymidine incorporation was measured in a liquid scintillation counter (model LS 3801, Beckman Instruments Inc). For proliferation assays, VSMCs were grown in 24-well plates. The cells were treated with mitogens in the presence or absence of AR inhibitors for 24 hours as described above, trypsinized, and counted with a hemocytometer.

HNE Metabolism

The [4-3H]HNE and its glutathione conjugates, glutathionyl-4-hydroxy nonenal (GS-HNE) and glutathionyl-1,4-dihydroxynone (GS-DHN), were synthesized and purified as described earlier.12 The human aortic VSMCs were seeded onto T75 flasks and cultured as above. When the cells were 80% confluent, the culture medium was removed, and the cells were washed 3 times with 20 mL of Krebs-Henseleit (KH) buffer containing (in mmol/L) NaCl 118, KCl 4.7, MgCl2 1.25, CaCl2 3.0, KH2PO4 1.25, EDTA 0.5, NaHCO3 25, and glucose 10, pH 7.4. Prewarmed (37°C) KH buffer had no observable effect on VSMC viability for the duration of the experiment. After 30 minutes of equilibration, [3H]HNE (103 cpm/nmol) was added to the medium at a final concentration of 50 nmol/L, and the cells were incubated at 37°C for an additional 30 minutes. After incubation, the medium was completely transferred into a syringe and passed through a 0.2-μm filter. The filtrate was then injected into a Nova-Pak C8 column (3.9×150 mm) mounted on an Alliance high-performance liquid chromatography (HPLC) system (Waters) with a 996-photodiode array detector. The metabolites of HNE were separated as described before.12

Electrospray Mass Spectrometry (ESI/MS)

ESI/MS analyses were performed on a single-quadrupole Micromass LCZ instrument. The operating parameters were as follows: capillary voltage, 2.9 kV; cone voltage, 26 V; extractor voltage, 4 V; source block temperature, 50°C; and desolvation temperature of 100°C. N2 at 3 psi was used as a nebulizer gas. Samples were lyophilized and resuspended in 0.1 mL of 50/50/0.5% (vol/vol%/%), acetonitrile/water/acetic acid and then introduced into the MS by using a Harvard syringe pump at a rate of 10 μL/min. Spectra were acquired with a scan time of 3.9 seconds and an interscan time of 0.1 second for a duration of 1 minute over 200 to 1000 AMU.

Rat Carotid Injury Model

In vivo proliferation of VSMCs was examined in the rat carotid artery model of restenosis as described previously.24 In brief, 12 adult, male Sprague-Dawley rats (400 to 500 g; Zivic Miller, Zelienople, PA) were anesthetized with an intraperitoneal injection of ketamine (2 mg/kg) and xylazine (4 mg/kg). The left internal carotid artery was then injured by balloon withdrawal 3 times, thus creating a denuded area. The right carotid artery remained uninjured and served as a control for each animal. Starting 1 day before injury and throughout the observation time, the animals were fed either the AR inhibitor sorbinil (40 mg·kg−1·d−1) or PBS (control). Sorbinil was brought in suspension in PBS by sonication and into aqueous solution by dropwise addition of 0.1 mol/L NaOH. There were no signs of toxicity related to drug administration. The carotid arteries were perfusion-fixed with 4% paraformaldehyde at 4, 10, or 21 days after injury. The tissues were dehydrated and stored in 70% ethanol. Cross sections obtained from injured regions were prepared. Slides were fixed and stained with hematoxylin and eosin as previously.
Immunohistochemistry

Carotid arteries were stored in 70% ethanol after fixation for 16 hours in 10% neutral buffered formalin. The tissue was embedded in paraffin, sectioned at 4 μm, floated on a protein-free water bath, and picked up on positively charged glass slides. The slides were air-dried overnight, oven-dried at 58°C for 1 hour, cooled to room temperature, heat-fixed in a microwave twice at 1 minute each, dewaxed in xylene, hydrated in alcohol, and placed in distilled water. The slides were then serially incubated in a 3% H2O2 1:100 dilution of immunoaffinity-purified rabbit anti-AR antibody raised against human AR for 45 minutes, LINK-rat (DAKO LABS2 rat kit) for 20 minutes, LABEL-Rat for 20 minutes, diaminobenzidine buffer for 10 minutes, and hematoxylin for 1 minute. After each incubation, the slides were rinsed with the buffer. The slides were then dehydrated, cleared in xylene, mounted in Pertmount, and photographed with a Nikon microscope.

Statistical Analysis

The data are expressed as mean±SEM. For multiple treatment groups, 1-way ANOVA followed by Bonferroni’s t test was applied (for in vitro data). For analysis of the in vivo data, a Mann-Whitney rank-sum test was used (SigmaStat, Jandel Scientific). A value of P<0.05 was considered statistically significant.

Results

In the initial set of experiments, changes in expression of the AR gene in response to several mitogens were examined. For these studies, growth-arrested human aortic VSMCs were exposed to serum (10%), bFGF (20 ng/mL), thrombin (2 U/mL), and the lipid peroxidation product HNE (2.5 μmol/L). After incubation for 8 hours, the steady-state levels of AR mRNA were determined by Northern blot analysis. As shown in Figure 1A, exposure to these reagents led to an increase in AR mRNA. Densitometric quantification with 18S rRNA as an internal control showed that the steady-state levels of AR mRNA were increased 2- to 4-fold on treatment with serum, bFGF, thrombin, and HNE. Analysis of the time course for the increase in the relative abundance of AR mRNA indicated a maximal effect (4-fold) after 8 hours of treatment with thrombin (data not shown). In similar experiments, the effect of mitogen treatment on the expression of AR protein was examined by Western blot analysis. Compared with untreated cells, there was a 4- to 7-fold increase in AR protein after treatment with serum, bFGF, thrombin, and HNE (Figure 1B). The increases in AR mRNA and protein were accompanied by a 2- to 3-fold increase in AR enzyme activity, as determined by using HNE as a substrate in homogenates of cells treated with bFGF and serum for 12 hours (data not shown). These data indicate that stimulation of VSMCs with mitogens that utilize distinct signaling pathways, such as tyrosine kinase (bFGF) and G protein–coupled (thrombin) receptors, leads to upregulation of AR.

To assess the role of AR in VSMC growth, we examined the effects of the AR inhibitors sorbinil and tolrestat. In the first series of experiments, growth-arrested VSMCs were incubated with various concentrations of sorbinil and tolrestat. When the cells were treated with 1 or 10 μmol/L tolrestat or sorbinil, no significant loss of viability was observed. However, 100 μmol/L sorbinil caused a 36% decrease in cell viability. Thus, subsequent experiments were restricted to the nontoxic concentrations of these inhibitors. Exposure to increasing concentrations of sorbinil decreased proliferation of serum-stimulated VSMCs, and ~60% inhibition of cell growth was observed at the highest nontoxic concentration of sorbinil (Figure 2A). Cell growth was also inhibited by tolrestat. Exposure to 10 μmol/L tolrestat led to a 60% to 80% decrease in VSMC proliferation (Figure 2B) and DNA synthesis (Figure 2C) in cells stimulated by serum, thrombin, or HNE. Taken together, these observations indicate that structurally unrelated inhibitors of AR prevent VSMC growth in response to diverse mitogenic stimuli.

Parallel studies were performed to examine the role of AR in VSMC metabolism of reactive aldehydes derived from lipid peroxidation. For these studies, we used HNE as a model aldehyde, because it is one of the most reactive and abundant end products of lipid peroxidation. On the basis of our previous studies, we expected the AR-catalyzed component of HNE metabolism to be the reduction of the glutathione conjugate of HNE (GS-HNE) to its corresponding alcohol (GS-DHN). To aid identification and characterization of these metabolites, GS-HNE and GS-DHN were synthesized as described in Methods. On ESI/MS, reagent GS-HNE showed a pseudomolecular [M+H]+ ion with a mass-to-charge ratio (m/z) of 464. Additional species with m/z values of 446 and 455 were also observed. The relative abundance of these ions varied with the cone voltage. At low cone voltages, the ions with low m/z values could be converted to 464, indicating that the 464- and 455-m/z ions were formed due to the loss of a single water molecule [M-18] from the monomeric and dimeric forms of the parent 464-m/z ion, respectively. For each experiment, the tune parameters were optimized for maximal sensitivity, which resulted in differential...
distribution of GS-HNE between these ions. For calculation of the total GS-HNE concentration, the peak intensities at 446 and 464 m/z were added to twice the value of the intensity at 455. The ESI/MS spectrum of reagent GS-DHN displayed a predominant peak at m/z 466 (data not shown). No daughter ions arising from the dehydration of this conjugate were observed under any of the conditions tested.

To examine the role of AR in HNE metabolism, the VSMCs in culture were exposed to [3H]HNE. After 30 minutes of incubation with the aldehyde, the radioactivity in the medium was separated by HPLC. The major radioactivity peak eluted with a retention time of 15 minutes, which was identical to the retention time of reagent GS-HNE. This peak accounted for 40% of the HNE metabolized. In control experiments, [3H]HNE incubated with the medium in the absence of cells was found to remain unchanged for a total observation time of 1 hour. Cellular metabolites of HNE separated by HPLC were pooled, lyophilized, and examined by ESI/MS. As shown in Figure 3A, the mass spectra of the peak containing the glutathione conjugates showed prominent
Confluent VSMCs were removed from culture and incubated with HNE. Conjugates corresponding to glutathione conjugates were pooled, lyophilized, and analyzed by ESI/MS as described in text. Note: y axes of the spectra are normalized to the intensity of the most abundant ion and, therefore, are not directly comparable.

The extent of GS-HNE to GS-DHN was determined in cells cultured with 0.1% or 10% serum or 0.1% serum with 100 µmol/L sorbinil. After 30 minutes of incubation, the media were separated by HPLC, and peaks corresponding to glutathione conjugates were pooled, lyophilized, and analyzed by ESI/MS as described in text. Note: y axes of the spectra are normalized to the intensity of the most abundant ion and, therefore, are not directly comparable.

**Figure 3. Involvement of AR in glutathione-linked metabolism of HNE.** Confluent VSMCs were removed from culture and incubated with KH buffer containing 50 nmol of [3H]HNE with (A) and without (B) 100 µmol/L sorbinil. After 30 minutes of incubation, the media were separated by HPLC, and peaks corresponding to glutathione conjugates were pooled, lyophilized, and analyzed by ESI/MS as described in text. Note: y axes of the spectra are normalized to the intensity of the most abundant ion and, therefore, are not directly comparable.

Conversion of GS-HNE to GS-DHN, if catalyzed by AR, should be greater in cells cultured with 10% than with 0.1% serum. As before, cells cultured in the presence of 10% serum showed a high extent of conversion of GS-HNE to GS-DHN. However, in cells cultured with 0.1% serum, the conversion of GS-HNE to GS-DHN was significantly attenuated. Calculations of peak intensities from 3 identical experiments showed that the extent of GS-DHN formation decreased from 49.2 ± 2.6% with 10% serum to 27.0 ± 6.6% in the presence of 0.1% serum (P < 0.05). These experiments showed that serum stimulation enhances the extent to which GS-HNE is converted to GS-HNE, consistent with the mitogenic upregulation of AR activity. Moreover, these data also indicated that the VSMC membrane is permeable to sorbinil and that at the concentrations used, the drug is pharmacologically active in these cells.

Because inhibition of AR prevents mitogen-induced VSMC proliferation in vitro, we examined the role of AR in VSMC growth contributing to neointimal formation after balloon injury to rat carotid arteries. Cross sections obtained from control (untreated) carotid arteries showed no significant staining of the medial SMCs, although the single epithelial layer and the surrounding adipocytes were intensely stained (Figure 4A). In contrast, sections of carotid arteries obtained 10 and 21 days after balloon injury showed intense staining associated with the neointima, whereas no staining was associated with the media (Figures 4B and 4C), indicating a specific association of AR with proliferating VSMCs.

Because inhibition of AR inhibited VSMC proliferation in culture and AR was upregulated in the proliferating neointima, we examined the effects of AR inhibitors on VSMC growth in vivo. For these experiments, sorbinil was used to inhibit AR, owing to its extensive evaluation in animal safety studies that resulted in no known health risks from laboratory exposure. Administration of sorbinil by oral gavage (40 mg · kg⁻¹ · d⁻¹) was started 1 day before balloon injury and was maintained throughout the observation period. A total of 12 animals were included in the study, 6 sham-treated controls and 6 animals treated with sorbinil. At day 4 after balloon injury, neointimal formation was minimal in both treatment groups, whereas marked intimal hyperplasia was observed at days 10 and 21 (Figure 5). Lesion size was quantified as the ratio of area of the neointima to that of the media. This ratio was 0.007 ± 0.001, 0.625 ± 0.015, and 1.45 ± 0.11 at 4, 10, and 21 days after injury and was reduced to 0.006 ± 0.001, 0.305 ± 0.01, and 0.6 ± 0.11, respectively. These values show a significant (P < 0.05) decrease in area of neointima to media in sorbinil-treated animals, resulting in a 51% and a 58% decrease in neointimal formation at days 10 and 21, respectively. On the basis of these data, we infer that inhibition of AR decreases intimal proliferation of VSMCs in balloon-injured rat carotid arteries.

**Discussion**

The results of this study suggest that AR, an enzyme involved in the metabolism of aldehydes generated by lipid peroxidation, is an important component of VSMC growth. Involvement of AR in cell growth is supported by the observations that stimulation of VSMCs by mitogens leads to upregulation of AR and that increased AR is associated with the proliferating cells of the neointima. Moreover, pharmacological...
inhibition of the enzyme prevents DNA synthesis and cell growth in culture and intimal hyperplasia in vivo. Because inhibition of AR was found to prevent glutathione-linked metabolism of the lipid peroxidation–derived aldehyde HNE, facilitation of cell growth by AR appears to be in part due to increased detoxification of aldehydes generated by mitogenic signaling involving ROS.

The specific association of AR with cell growth is suggested by our observation that quiescent cells of the media in uninjured carotid artery do not stain with anti-AR antibody. This observation is consistent with previous immunohistochemical studies that showed that in rat blood vessels, AR is localized exclusively to the endothelial lining of the aorta and the muscular arteries with no significant expression in the media. As in our study (Figure 5), intense staining was found to be associated with adipocytes found in the adventitia bordering the vessel. Similarly, Rittner et al reported a complete absence of AR-specific transcripts and the virtual absence of staining with anti-AR antibody in normal human arteries. The absence of AR in quiescent VSMCs and its high expression in VSMCs proliferating in culture suggest that the enzyme is specifically upregulated during growth. The association of AR with cell growth is further supported by the observation that after balloon injury, the proliferating cells of the neointima showed high levels of expression of this enzyme. The formation of protein-HNE adducts localized to the proliferating neointima and inflamed arteries associated with increased expression of AR suggests a critical link between oxidative stress, AR, and cell growth.

The upregulation of AR during VSMC growth in culture appears to be due to signaling mechanisms that converge to common downstream mediators. We found that agonists of both tyrosine kinase (bFGF) and G protein–coupled (thrombin) receptors stimulate AR. Because both FGF and thrombin generate ROS, it is likely that upregulation of AR during growth is due to increased ROS generation. Direct stimulation of VSMCs with H2O2 or HNE also enhances AR (Reference 32 and Figure 1 of the present study), and the enzyme is also upregulated by tumor necrosis factor-α and interferon-γ, cytokines that are known to generate ROS. During giant-cell arteritis, the expression of AR is increased in T cells, macrophages, and VSMCs in areas of high oxidative stress and HNE formation. Interestingly, an AR-related protein (YBR49W) is markedly induced as part of the yeast adaptive response to H2O2, in which it has been suggested to be required for scavenging bioactive aldehydes derived from lipid peroxidation. Thus, the sensitivity of AR to oxidants appears to be a phylogenetically well conserved response and may be due to the presence of consensus sequences for binding of ROS-sensitive transcription factors such as nuclear factor-κB and activator protein-1 in the promoter site of the gene. Increased transcription of AR by tumor necrosis factor-α has been recently demonstrated to be mediated by nuclear factor-κB. Although the role of specific transcription factors in mitogenic stimulation of AR was not tested, our results are consistent with redox regulation of the AR gene and indicate that AR may be upregulated in part by ROS generated by growth factors.

The redox sensitivity of the AR gene is consistent with the involvement of AR in the metabolism of aldehydes derived...
from lipid peroxidation. Although this enzyme has been studied mostly within the context of hyperglycemic injury, recent evidence shows that in vitro, AR and the closely related murine aldo-keto reductase FR-1 are efficient catalysts for the reduction of medium-chain hydrophobic aldehydes. In most cells, these aldehydes are derived from ROS-mediated peroxidation of unsaturated lipids of membranes and lipoproteins such as LDL. Owing to their high reactivity, lipid peroxidation-derived aldehydes have been suggested to be second messengers of ROS. Low concentrations of HNE are mitogenic for VSMCs, whereas high concentrations of HNE and related aldehydes elicit a variety of cytotoxic effects. Thus, processes that metabolize aldehydes are likely to be key determinants of their mitogenic and cytotoxic effects.

We found that in VSMCs, inhibition of AR prevents the formation of GS-DHN from HNE. Because DHN itself is not electrophilic and does not directly conjugate with GSH, the formation of GS-DHN appears to be generated by the AR-catalyzed reduction of the parent GS-HNE conjugate. Several lines of evidence support the view that this reduction is a critical determinant of the cellular effects of HNE. It has been shown that inhibitors of AR exacerbate HNE toxicity to VSMCs, enhance the formation of protein-HNE adducts, and promote oxidative stress–induced apoptotic cell death in vivo. In addition, increased expression of AR in VSMCs exposed to HNE (Reference 13 and Figure 1) further suggests that AR is an important component of HNE metabolism in these cells. Thus, by participating in the metabolism and removal of lipid-derived aldehydes, AR could facilitate cell growth by regulating the cellular reactivity of ROS and their products and minimizing oxidative stress generated by growth factors and cytokines. Although this is expected for growth factors such as thrombin and FGF that generate high levels of ROS, it is somewhat surprising that serum-induced growth was also prevented by inhibiting AR. It is possible that inhibition of AR enhances oxidative stress, regardless of whether ROS constitute part of the mitogenic signaling or are generated intrinsically by the high metabolic activity of growing cells. Alternatively, products of AR catalysis may be direct stimulants of cell growth. Further investigations are, therefore, required to distinguish between these possibilities.

Prevention of VSMC growth by inhibiting AR suggests a new therapeutic approach to treat intimal hyperplasia during restenosis. Because abnormal proliferation of VSMCs is also a critical contributing factor to other vascular disorders, such as atherosclerosis, hypertension, and vein graft disease, inhibition of AR may be useful also for the treatment of several related clinical states. Although many strategies have been...
devised to prevent VSMC proliferation, most have been targeted to inhibit either individual growth factors or the second-messenger systems that transduce their mitogenic signals. However, redundancy of growth factors within the vessel wall limits the efficacy of interrupting specific mitogens. Moreover, the second messengers of growth factors (eg, tyrosine kinases and G protein), as well as the immediate-early responses they trigger, are common to a variety of stimuli and regulate a wide array of cellular processes. Because of such pleiotropic effects, the toxicity associated with inhibitors of these messengers (eg, tyrosine kinase inhibitors) is expected to be high. In contrast, because it generates cell-specific metabolites, the delayed-early gene response is likely to be less redundant. Thus, inhibition of AR may be particularly useful, because this enzyme is specifically induced during VSMC growth and inhibition of the enzyme prevents cell growth stimulated by several different mitogenic stimuli. However, the effects of ROS in general and HNE in particular are concentration dependent, stimulating cell growth at low concentrations and promoting cell death at higher concentrations (vide supra). Hence, the outcome of inhibiting AR cannot be readily predicted, since this may lead to increased cell death or growth, depending on the intrinsic levels of oxidative stress. Therefore, further studies are needed to establish the therapeutic efficacy of this class of drugs for the management of restenosis, atherosclerosis, and other vasculoproliferative disorders.

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