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Differential effect of simvastatin on activation of Rac_1 vs. activation of the heat shock protein 27-mediated pathway upon oxidative stress, in human smooth muscle cells

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Abstract

In the present study, we have analyzed the response of human smooth muscle cell (SMC)s to oxidative stress, in terms of recruitment of key elements of the stress-activated protein kinase (SAPK) pathway, such as Rac_1, p38, and the small heat shock protein (HSP)27. The level of expression of three small HSPs, αB-crystallin, HSP20, HSP27, as well as the phosphorylation levels of HSP27 and p38, were higher in cultured, asynchronously growing SMCs originating from left interior mammary artery (LIMA) than those originating from aorta, saphenous vein, and umbilical vein, validating the choice of SMCs from LIMA as a model system in our study. In synchronized, quiescent SMCs from LIMA, oxidative stress (H_2O_2 stimulation)-induced membrane translocation of Rac_1, p38 phosphorylation, membrane translocation, and phosphorylation of HSP27. In these cells, simvastatin (S), an HMG-CoA reductase inhibitor, blocked, in a mevalonate-dependent way, oxidative stress-induced membrane translocation of Rac_1. However, S pretreatment prior to oxidative stress increased the levels of p38 phosphorylation, HSP27 membrane translocation/phosphorylation, actin polymerization, and apoptosis in these cells, in a mevalonate-dependent way. These results establish that S pretreatment has a stimulatory effect on the stress-activated p38/HSP27 pathway, despite its blocking effect on Rac_1 activation.

Keywords: Human smooth muscle cells; Simvastatin; p38; Oxidative stress; Rac_1; HSP27; Actin polymerization; Apoptosis

1. Introduction

Vascular SMCs are a major constituent of the blood vessel wall, responsible for the maintenance of vascular structure and function. The arterial wall is continually remodeling, in response to various stressors, such as hemodynamic stress (narrowing of the lumen of the artery) or oxidative stress (oxLDL and reactive oxygen species (ROS)). In these conditions, a dynamic reorganization of the vascular cytoskeleton must occur to facilitate alterations in vascular cell shape, cell migration or cell proliferation.

After myocardial ischemia or oxidative injury, small and large HSPs (from small and large molecular weight, respectively) are engaged in a cascade of events leading ultimately to cell survival or cell death. HSP70, from the large HSP family, is one of the best known and characterized HSPs and has been presented as a cytoprotective agent during atherosclerosis ([1], for a review). Its synthesis can be induced by mechanical forces [2] or oxidized lipoproteins [3]. Unlike the ubiquitous HSP70, specific members

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Abbreviations: AO, aorta; ERK, extracellular-regulated protein kinase; FACS, fluorescence-activated cell sorting; FCS, fetal calf serum; H_2O_2, hydrogen peroxide; HS, human serum; HSP, heat shock protein; JNK, jun-activated protein kinase; LIMA, left interior mammary artery; M, mevalonate; MAPK, mitogen-activated protein kinase; NaArs, sodium arsenate; oxLDL, oxidized low density lipoprotein; PBS, phosphate buffer saline; POD, peroxidase; ROS, reactive oxygen species; RT, room temperature; S, simvastatin; SAPK, stress-activated protein kinase; SMC, smooth muscle cell; SV, saphenous vein; TBS, Tris-buffered saline; UV, umbilical vein.

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of the small HSP family, such as HSP27, αB-crystallin or HSP20, exhibit tissue-restricted expression, suggesting potential specialized properties in the cardiovascular system. p38/SAPK2 phosphorylation, followed by HSP27 phosphorylation, was shown to regulate actin polymerization upon oxidative [4] or thermal shock [5]. These events may contribute to a better maintenance of actin filament integrity leading to cell survival and prevention of apoptosis ([1], for a review).

Beneficial pleiotropic effects of statins on the artery wall, apart from their cholesterol-lowering property, have been supported by some studies [6]. In PDGF-stimulated, SMCs *in vitro*, the anti-proliferative effect of S can be explained, not only through the blockage of RhoA-mediated signaling events [7,8], but also Rac_1-mediated signaling events [8]. Moreover, lovastatin, another statin, can induce the depolymerization of actin cables in a fibroblastic-type cell line, under certain conditions [9]. Very little information on the effect of S on stress/inflammatory pathways has been collected until now. We have, therefore, analyzed the effect of this drug on the activation of Rac_1, p38, and HSP27 in oxidatively stressed HSMCs *in vitro*.

2. Materials and methods

2.1. Materials

The S was kindly provided by Sankyo Co. The drug was in the open sodium salt form and kept at −20° as a 10 mM stock solution (in 100% ethanol) up to 2 weeks. SB203580 (Alexis Corp.) was kept at −20° as 100 mM (in 100% DMSO) stock solution. Mevalonate (M), sodium arsenate (NaAs), and hydrogen peroxide (H_2O_2) were purchased from Sigma–Aldrich. Polyclonal peroxidase-conjugated, anti-rabbit immunoglobulin G (anti-rabbit IgG-POD) and protease inhibitor cocktail tablets (Complete^{TD}) were from Roche B.V. Rabbit polyclonal immunoglobulin G anti-ERK1&2 (anti-ERK1&2), rabbit polyclonal immunoglobulin G anti-RhoA (anti-RhoA), rabbit polyclonal immunoglobulin G anti-p38 (anti-p38), goat polyclonal immunoglobulin G anti-actin (anti-actin), goat polyclonal immunoglobulin G anti-HSP27 (anti-HSP27), and polyclonal peroxidase-conjugated, anti-mouse immunoglobulin G (anti-mouse IgG-POD) were from Santa Cruz Biotech., Inc. Rabbit polyclonal immunoglobulin G anti-phosphorylated p38 (anti-phospho p38) was from New England Biolabs Inc. Mouse polyclonal immunoglobulin G anti-Rac_1 (anti-Rac_1) was from Transduction Lab. The anti-αB-crystallin and anti-HSP20 antisera were raised in rabbits against, respectively, purified bovine lens αB-crystallin and recombinant rat HSP20 [10]. The anti-phosphorylated HSP27 (anti-phospho HSP27), affinity purified antibody was raised in sheep against the Ser78-phosphorylated sequence of human HSP27 [11]. Rhodamin-phalloidin was from Molecular Probes Europe BV. Polyclonal peroxidase-conjugated, anti-goat immunoglobulin G (anti-goat IgG-POD) was from Nordic Immunology. Polyclonal peroxidase-conjugated, anti-sheep immunoglobulin G (anti-sheep IgG-POD) was from Pierce Inc.

2.2. SMC cultures

Human SMCs were isolated from LIMA, aorta (AO), saphenous vein (SV) explants or umbilical vein (UV) from human donors. Vascular specimens, discarded after the operation, were kindly provided by the workers from the Leiden University Medical Center, according to rules of the local ethical committee. The cells were cultured as previously described [12]. SMC cultures were used within six passages in this study. The average doubling time was 2.5 days.

Cells were seeded in culture wells in Dulbecco's modified Eagle's medium (Invitrogen) containing 10% fetal calf serum (FCS) and 10% HS. Unless otherwise mentioned, the cells were made quiescent 24 hr later by replacing the medium with Dulbecco's modified Eagle's medium containing 0.4% FCS, for 2.5 days. Quiescent cells were then incubated as described in the figure legends.

2.3. Measurement of membrane translocation by subcellular fractionation

This technique was performed according to Hirai *et al.* [13]. Incubated cells (200,000 cells per 10-cm^2 well; 2 wells per sample) were washed in ice-cold phosphate buffer saline (PBS), disrupted by incubation (30 min, 4°) in hypotonic buffer (5 mM Tris–HCl, 5 mM NaCl, 1 mM $CaCl_2$, 2 mM EGTA, 1 mM $MgCl_2$, 2 mM DTT; pH 7.0), containing a mixture of protease inhibitors (Complete^{TD}), and separated by centrifugation (100,000 *g*, 1 hr at 4°) in membrane/cytoskeleton-associated and cytosolic fractions. The volume of each fraction was adjusted to comparable values, diluted in SDS–PAGE sample buffer (3.3% SDS, 5% β-mercaptoethanol, 137 mM Tris–phosphate (pH 6.7), 10% glycerol), and specific proteins contained in the membrane/cytoskeleton-associated fractions were detected by western blotting.

2.4. Western blot analysis

Cells were lysed in SDS–PAGE sample buffer and processed for SDS–PAGE analysis. Proteins were resolved on precast 10% polyacrylamide mini gels (Gradipore Ltd.) and transferred onto Polyvinylidene Fluoride membranes (0.45 μm; Millipore Corp.). The membranes were first shaken for 1 hr in Tris-buffered saline (TBS, 20 mM Tris, 136 mM NaCl, pH 7.6) containing 0.25% Tween 20 and 5% nonfat, protein-enriched milk (Protifar^{TD}), then incubated with anti-HSP20, anti αB-crystallin (1/2000 dilution in TBS/Tween, overnight at 4°), anti-HSP27, anti-p38,

anti-phospho p38, anti-RhoA or anti-Rac_1 (1/1000 dilution in TBS/Tween, overnight at 4°), followed by an incubation with an anti-rabbit IgG-POD (1/1000 dilution in TBS/Tween, 1 hr at room temperature (RT)) or with an anti-mouse IgG-POD (1/2000 dilution in TBS/Tween, 1 hr at RT). When the anti-phospho p38 antibody was used, vanadate (0.5 mM) was present in all incubations in order to prevent phosphatase activity. The immunoreactive proteins were visualized by chemiluminescence using a peroxidase enzymatic reaction (enhanced chemoluminescence kit, Amersham). The results of one experiment, representative for a series of three to four experiments are shown.

2.5. Fluorescence microscopy

Quiescent cells were seeded in Costar® Special Optics-96 well black plate with ultra-thin clear bottom, precoated with 1% gelatin (6000 cells per well). They were incubated and stimulated as described in the legend of the corresponding figure, washed twice with PBS, and fixed in 3% paraformaldehyde/PBS for 30 min at RT. After washing twice in 50 mM glycine/PBS for 5 min at RT, cells were permeabilized in 0.2% Triton X-100 for 5 min at RT. After washing again in 50 mM glycine/PBS, 2.5 μL rhodamin-phalloidin (0.5 units) were added per well and the incubation continued for 60 min at RT, in the dark. After a few washings in PBS, dried wells were observed up-side down using a Nikon Microphot-FXA fluorescent microscope and cells were photographed using a digital camera.

2.6. Apoptosis assay

Apoptosis was determined by fluorescence-activated cell sorting (FACS), according to van de Water *et al.* [14], except that the cells were harvested by 0.05% collagenase treatment. The number of cells present in sub-G_0/G_1 was calculated using the Cell Quest software (Becton Dickinson). Quantitative results are given as mean ± SD.

3. Results

3.1. Expression of small HSPs in cultured SMCs isolated from various tissues: predominance of the p38/SAPK2 pathway in mammary artery

Cultured, asynchronously growing (in presence of 10% HS and 10% FCS), HSMCs isolated from AO, LIMA, SV, and UV were compared for the expression of the three small HSPs, HSP27, αB-crystallin, and HSP20 (Fig. 1A). Interestingly, SMCs from LIMA displayed the highest amount of the three small HSPs, suggesting a more prominent physiological role for these proteins in these cells, compared to those from other origins. HSP27 expression levels were the lowest in SMCs from SV, whereas αB-crystallin expression was almost absent in SMCs from UV.

In these culture conditions, HSP27 phosphorylation state was the highest in SMCs from AO and p38 phosphorylation state was the highest in SMCs from LIMA. However, both high levels of p38/SAPK2 phosphorylation and HSP27 phosphorylation were observed in SMCs from LIMA (Fig. 1B and C), whereas the total expression of p38 (Fig. 1C) and actin (Fig. 1D) was similar between SMCs from various origins. These data show that the SAPK/stress pathway is significantly activated in asynchronously growing SMCs isolated from LIMA, compared to SMCs isolated from other vascular tissues. Therefore, SMCs from LIMA were chosen as an experimental model in this study in order to analyze the mechanism of the oxidative stress response in quiescent SMCs and the effect of S on this process.

3.2. Rac_1, p38/SAPK2, and HSP27 activation by oxidative stress in human SMCs from LIMA

During cellular stress, the p38/SAPK2 pathway is normally recruited and it involves the participation of Rac_1 and HSP27 activation, as upstream and downstream steps, respectively. This was assessed by measuring membrane translocation of Rac_1, p38 phosphorylation, membrane/cytoskeleton translocation, and phosphorylation of HSP27 upon stress, in quiescent SMCs. Very low levels of membrane-associated Rac_1, p38, and HSP27 phosphorylation and membrane/cytoskeleton-associated HSP27 were detected in control, nonstimulated cells (Figs. 2 and 3), indicating that activation of these proteins is cell-cycle dependent, as mentioned previously for PDGF-induced phosphorylation of p38 in these cells [8]. Oxidative (4 mM H_2O_2) and/or chemical (0.2 mM NaArs) stress increased the levels of membrane-associated Rac_1 (Fig. 2), with the most dramatic effect obtained with H_2O_2. Short-term phosphorylation of p38 and of HSP27 were observed after oxidative stress (Fig. 3A and C). Consistently, remarkably increased levels of membrane/cytoskeleton-associated HSP27 were detected after H_2O_2 stimulation, not after NaArs stimulation. Total levels of Rac_1 and HSP27 remained unchanged by these stress treatments (Figs. 2 and 3B).

3.3. Influence of S on Rac_1, p38/SAPK2, and HSP27 activation in human SMCs from LIMA

Protein prenylation is essential for the attachment to the plasma membrane of small GTPases, such as Rac_1, and, therefore, for its membrane translocation. We previously showed that S, an HMG-CoA reductase inhibitor, is able to block PDGF-induced membrane translocation of RhoA and Rac_1 in these cells [8]. In the present study, we observed an inhibitory effect of S on the membrane translocation of Rac_1 upon oxidative stress and that M co-addition could prevent this blockage (Fig. 4). The effect of S pretreatment was then further examined on the p38/

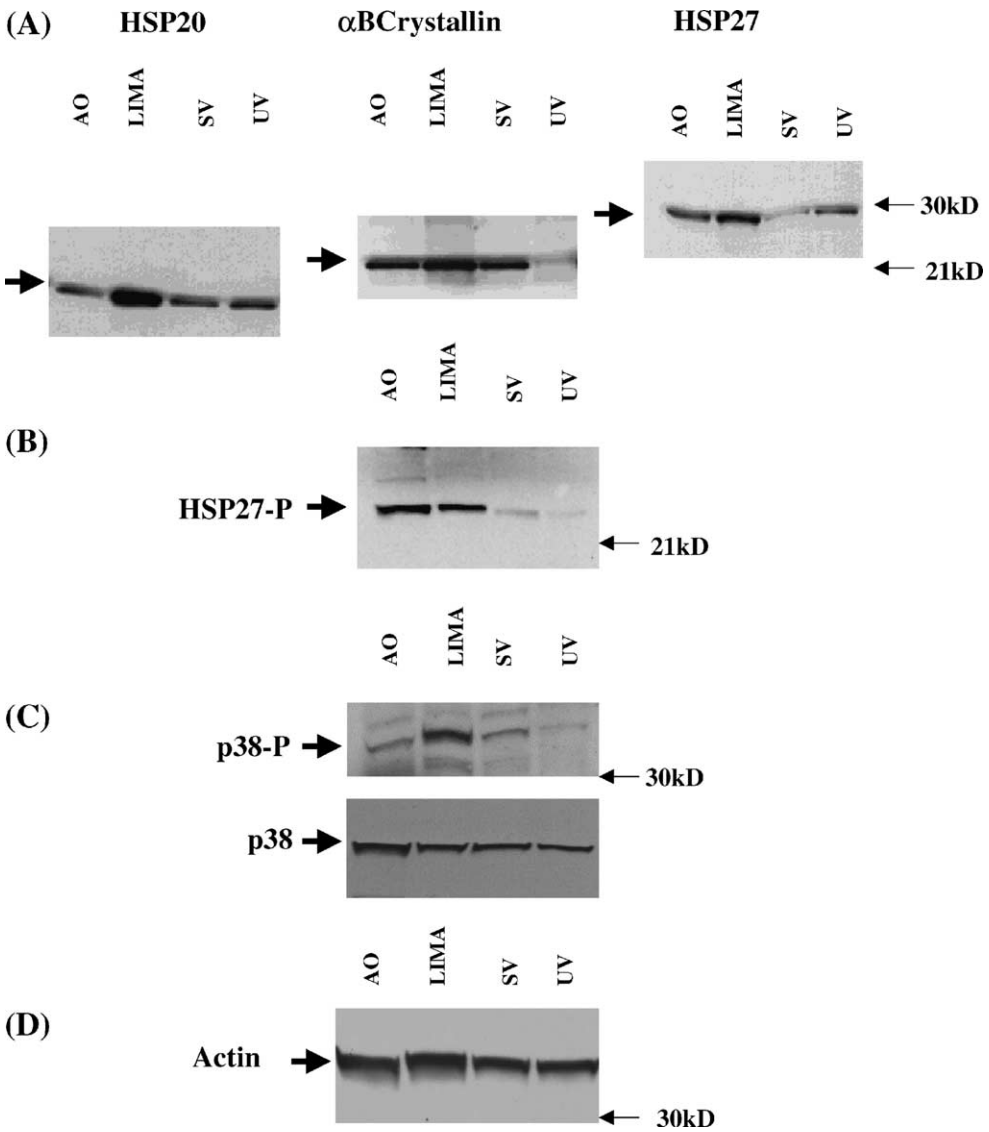

Fig. 1. Expression of HSP20, αB-crystallin, and HSP27 in human SMCs isolated from various tissues (A). Comparison of levels of HSP27 phosphorylation (B) and p38 phosphorylation (C) among these cells. Exponentially growing SMCs isolated from AO, LIMA, SV, and UV were lysed in H_2O diluted in SDS sample buffer, and the samples (52,000 cells per lane) were processed for western blot analysis using anti-HSP27, anti-αB-crystallin, and anti-HSP20 (A); anti-phosphorylated HSP27 (B); anti-phosphorylated p38 and anti-p38 (C) or anti-actin (D) antibodies. The results of an experiment, representative for three separately performed experiments are shown. Left arrow indicates the specific band for each blot. HSP27-P: phosphorylated HSP27; p38-P: phosphorylated p38. Right arrow indicates molecular weight markers.

SAPK2 pathway. Interestingly, oxidative stress-induced p38 phosphorylation was enhanced by S pretreatment (Fig. 5A). Similarly, membrane/cytoskeleton translocation and phosphorylation of HSP27, induced by oxidative stress, were enhanced by S pretreatment (Fig. 5B and C). Co-addition of M prevented these potentiating effects (Fig. 5A–C), indicating that isoprenylated proteins are probably involved in this process. As expected,

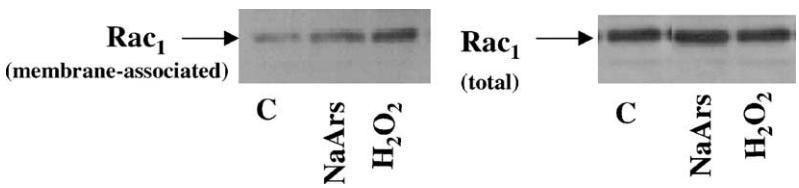

Fig. 2. Membrane translocation of Rac_1 upon stress in human SMCs from LIMA. Synchronized, quiescent human SMCs (400,000 cells per lane) were incubated with medium C, NaArs (200 μM) or H_2O_2 (4 mM) for 60 min at 37°, then processed for subcellular fractionation in order to isolate the membrane-associated fraction from the total cell lysate. Membrane and total fractions were processed directly for SDS–PAGE and western blotting was performed with anti-Rac_1. The results shown are representative for a series of four experiments.

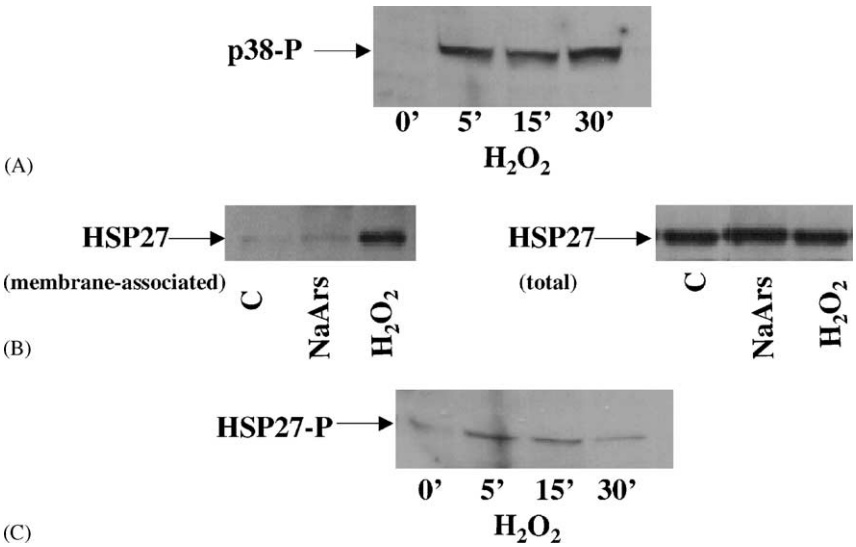

Fig. 3. p38 phosphorylation (A), membrane translocation (B), and phosphorylation (C) of HSP27 upon stress in human SMCs from LIMA. (A) Synchronized, quiescent human SMCs (50,000 cells per lane) were incubated with H_2O_2 (4 mM) at 37°, for the times indicated, and processed for western blotting analysis using anti-phosphorylated p38. (B) Synchronized, quiescent human SMC (400,000 cells per lane) were incubated with medium C, NaArs (200 μM) or H_2O_2 (4 mM) for 60 min at 37°, then processed for subcellular fractionation in order to isolate the membrane-associated fraction from the total cell lysate. Membrane and total fractions were processed directly for SDS–PAGE and western blotting was performed with anti-HSP27. (C) Synchronized, quiescent HSMC (50,000 cells per lane) were incubated with H_2O_2 (4 mM) at 37°, for the times indicated, and processed for western blotting analysis using anti-phosphorylated HSP27. The results are representative for a series of three experiments. P38-P: phosphorylated p38; HSP27-P: phosphorylated HSP27.

SB203580, a specific inhibitor of p38 mitogen-activated protein kinase (MAPK), inhibited oxidative stress-induced phosphorylation of p38 (Fig. 5A) and of HSP27 (Fig. 5C), confirming the sequential activation of these proteins.

It is important to note that the increase in p38/SAPK2 pathway activation, resulting from S pretreatment, could proceed in the absence of the membrane translocation of Rac_1 (Fig. 4).

3.4. Actin stress fiber formation in human SMCs from LIMA upon oxidative stress: influence of S on this process

Since activation of the p38/SAPK2 pathway is known to result in actin polymerization, quiescent SMCs from LIMA were stained with rhodamine-phalloidin, a specific marker of filamentous actin (as in actin cables), in order to assess the formation of actin stress fibers upon oxidative stress. As shown in Fig. 6A, control SMCs exhibited a dense polymerized actin network. Upon oxidative stress, a more intense labeling of actin cables was detected, indicating that additional actin polymerization had occurred (Fig. 6B). In presence of S, labeled actin cables increased and thickened into bundles (Fig. 6C), suggesting that S pretreatment is potentiating actin stress fiber formation. In presence of M, the S effect was almost totally prevented (Fig. 6D), with actin polymerization levels equivalent to those in non-S-treated, H_2O_2-stimulated cells.

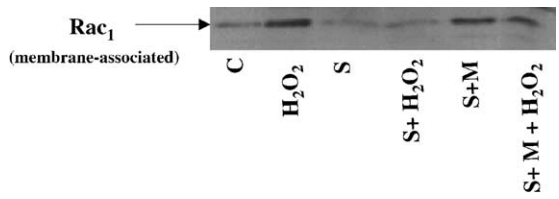

Fig. 4. Influence of S pretreatment on membrane translocation of Rac_1 upon stress in human SMCs from LIMA. Synchronized, quiescent human SMCs (400,000 cells per condition) were pretreated for 24 hr with S (10 μM) or S + M (10 and 200 μM, respectively). Cells were then incubated with medium C or H_2O_2 (4 mM), for 60 min at 37°, and processed as described in the legend of Fig. 2. The data shown are representative for four experiments.

3.5. Effect of oxidative stress on apoptosis induction in human SMCs from LIMA: influence of S on this process

In order to investigate the potential role of HSP27, an actin polymerization modulator, in cell survival or apoptotic pathways, the number of apoptotic cells (apoptotic level) was quantified by FACS analysis, in our experimental conditions (Fig. 7). Quiescent, as well as oxidatively stressed, human SMCs display the same low apoptosis level. In presence of S, slightly higher levels of apoptotic cells were observed in nonstimulated cells. Furthermore, when S-pretreated cells were submitted to oxidative stress (H_2O_2), apoptosis was induced, indicating that in stressed cells displaying a low level of apoptosis, addition of S (10 μM) could trigger the mechanism of programmed cell death. The almost total prevention of this effect by M co-addition reflects the involvement of prenylated proteins in this process.

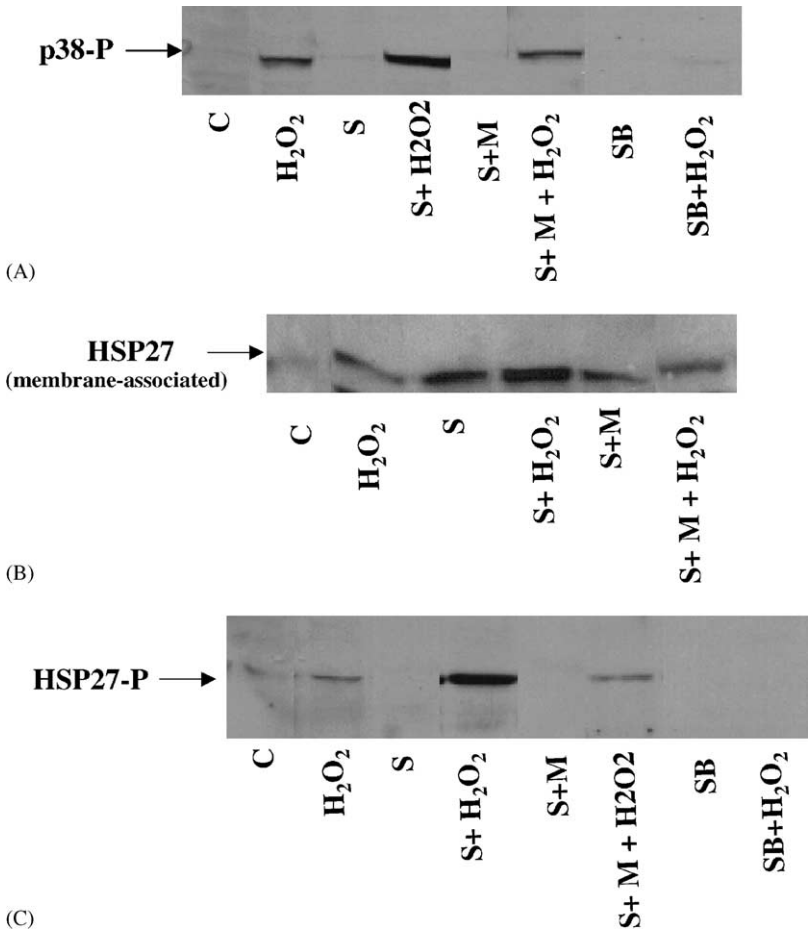

Fig. 5. Influence of S pretreatment on phosphorylation of p38 (A), membrane translocation (B), and phosphorylation (C) of HSP27 upon stress, in human SMCs from LIMA. Synchronized, quiescent human SMCs (400,000 cells per condition) were pretreated for 24 hr with S (10 μM), S + M (10 and 200 μM, respectively), or pretreated for 1 hr with SB203580 (SB, 3 μM). Cells were then incubated with medium C or H_2O_2 (4 mM) for 60 min at 37°, and processed as described in the legend of Fig. 3. The results of one experiment, representative for a series of three experiments are shown. p38-P: phosphorylated p38; HSP27-P: phosphorylated HSP27.

4. Discussion

In order to choose the best model system to investigate the p38/SAPK2 pathway and small HSPs in human SMCs, we compared the level of expression of three small HSPs, HSP27, αB-crystallin, and HSP20, in SMCs isolated from four different human origins: AO, LIMA, SV, and UV. SMCs from LIMA express a much higher amount of HSP20 and HSP27 than the SMCs from the other origins. Furthermore, phosphorylation of HSP27 and p38, both key elements of the SAPK2 pathway, were present at their highest levels in SMCs from LIMA. Therefore, all subsequent experiments were performed in these cells. αB-crystallin and HSP27 are potential candidates for the "first line of defense" against nonlethal stress ([1], for a review) and ectopic expression of HSP27 in cell lines was shown to contribute to a better maintenance of actin filament integrity and overall cell survival [5]. Moreover, human SMCs from mammary artery display a lower proliferative response than SMCs from SV, after pulsatile stretch [15] or growth factor stimulation [16]. All these facts, in combination with our data, strongly suggest that SMCs from mammary artery exhibit a higher level of cellular resistance to various stress factors, e.g. oxidative stress, which may contribute to a better potency of mammary artery grafts vs. SV grafts for by-pass surgery interventions.

Atherosclerosis is caused by an unchecked chronic inflammatory process, which involves the interaction of cells of the artery wall with oxLDL, ROS, and various inflammatory cells [17]. ROS, such as superoxide or H_2O_2, are important signaling molecules in cardiovascular cells and serve as second messengers to activate various proteins, such as p38/SAPK2 [18]. In the present study, we investigated in human SMCs the influence of H_2O_2-mediated oxidative stress on Rac_1 activation, p38 activation, HSP27 activation, actin polymerization, and apoptosis, in order to evaluate the impact of S pretreatment on these processes. Rac_1, a member of the Rho GTPase family, has been reported as an activator of the MAPK pathways recruited during cellular stress, such as JNK and p38/SAPK2 pathways. However, the exact role of this

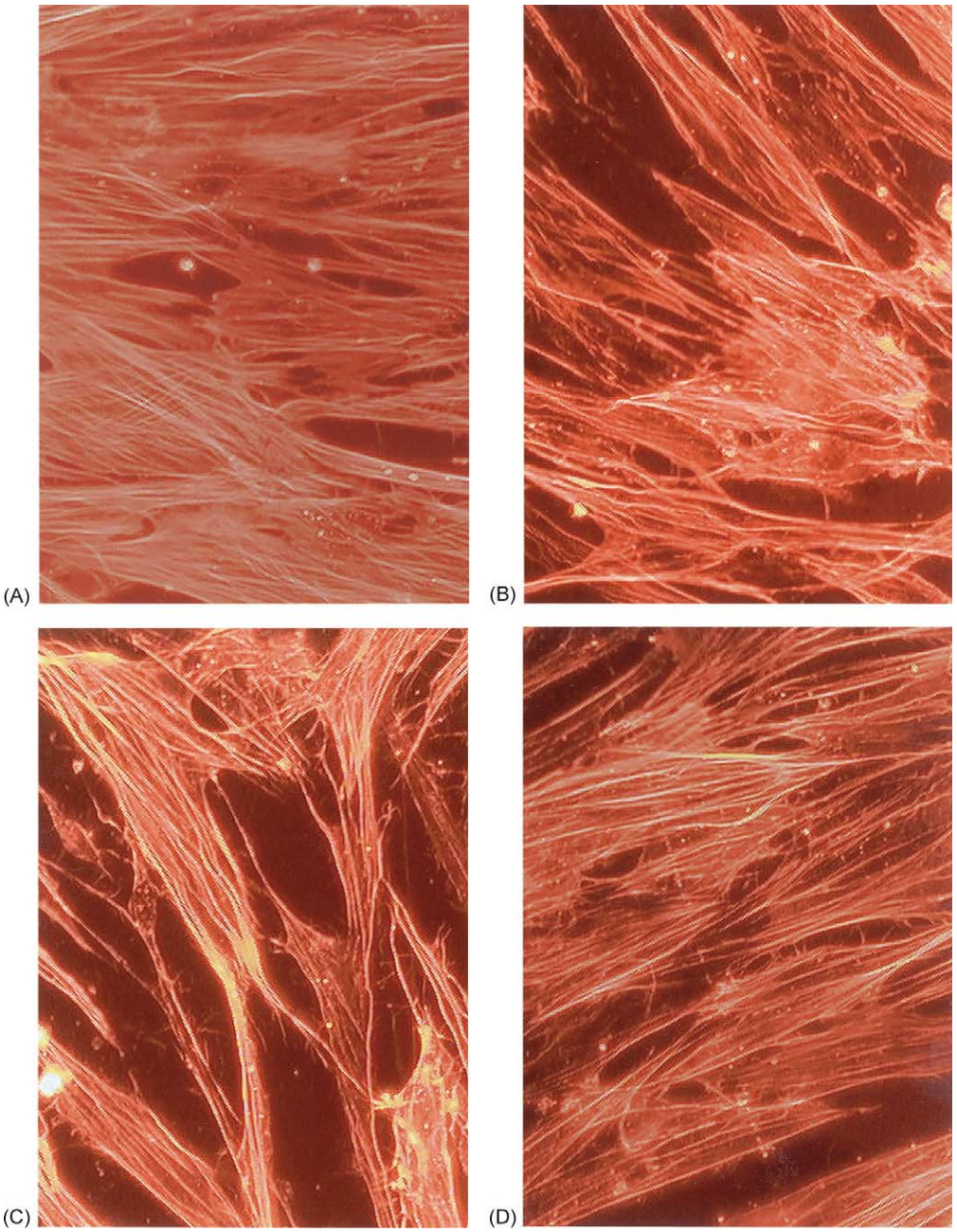

Fig. 6. Influence of S pretreatment on actin stress fiber formation upon oxidative stress, in human SMCs from LIMA. Synchronized, quiescent human SMCs (6000 cells per well) were pretreated for 24 hr with medium (A and B), S (10 μM) (C) or S + M (10 and 200 μM, respectively) (D). Cells were then incubated with medium (A) or H_2O_2 (4 mM) (B–D) for 60 min at 37°, washed, fixed, permeabilized, and incubated with rhodamin-phalloidin (0.5 units per well) for 60 min at RT, in the dark. After a few washings in PBS, dried wells were inspected up-side down using a Nikon Microphot-FXA fluorescent microscope and cells were photographed using a digital camera. The results shown are representative for three independently performed experiments. Magnification: ×260.

small GTPase in MAPK activation remains unclear since many experimental data have been obtained so far using overexpression of constitutively activated Rac or dominant negative Rac in transfected model systems [19]. In human SMCs from LIMA, oxidative stress induced membrane translocation of Rac_1. P38/SAPK2 is mostly recognized as a stress-sensitive kinase whose activation leads, directly or indirectly, to phosphorylation and activation of a number of transcription factors and cytoplasmic proteins, such as HSP27 [20]. The role of p38/HSP27 pathway has been described in the stimulation of SMCs by various ligands [21–24], resulting in cell contraction and/or migration. However, it is the first time, to our knowledge, that the effect of oxidative stress has been investigated in human SMCs. In our study, oxidative stress induced rapid p38 phosphorylation, HSP27 phosphorylation, and translocation to the cytoskeletal/membrane fraction. Interestingly, S pretreatment potentiated H_2O_2-induced p38 and HSP27 activation, despite its blockage of Rac_1 activation. This finding indicates that, in this system, there is no direct

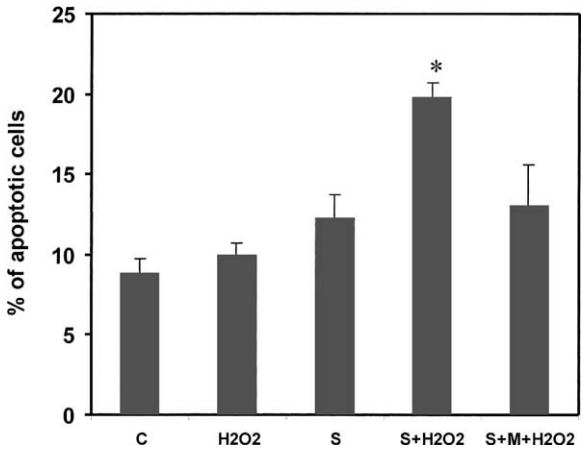

Fig. 7. Influence of S pretreatment on apoptosis induction, upon oxidative stress, in human SMCs from LIMA. Synchronized, quiescent human SMCs (150,000 cells per well) were pretreated for 24 hr with medium, S (10 μM) or S + M (10 and 200 μM respectively). Cells were then incubated with medium C or H_2O_2 (4 mM), for 60 min at 37° and DNA fragmentation was determined by FACS analysis. The percentage of apoptotic cells has been depicted. Bars represent SD (N = 3). $^*P < 0.05$, significantly different from control (C).

relationship between Rac_1 activation and the p38/HSP27 pathway and underlines the potential role of other S-sensitive, prenylated proteins, in the regulation of this pathway.

Both major vascular cell types, such as endothelial cells and SMCs, express either naturally or after stress high levels of HSP27 ([20], for a review). Therefore, in these cells, the p38/SAPK2 pathway is suspected to play a major role in modulating actin dynamics upon oxidative stress. In oxidatively stressed endothelial cells, reorganization of actin monomers (F-actin) into long trans-cytoplasmic stress fibers occurred within 10–15 min ([20], for a review) and it was demonstrated that this process was mediated by activation of p38/SAPK2 and HSP27. In human SMCs from LIMA, we observed that oxidative stress induced F-actin remodeling, with the formation of actin stress cables within a dense polymerized actin filament network, already present in quiescent cells. In the same cells, SB203580, which totally blocked p38 and HSP27 phosphorylation, partially prevented H_2O_2-induced actin polymerization (not shown), indicating that this process is mediated through the SAPK2 pathway. S pretreatment, which enhanced H_2O_2-induced p38 and HSP27 activation, consistently reinforced the actin stress fiber formation upon oxidative stress, in an M-dependent manner. This new finding suggests a preconditioning role for S in the induction of the heat shock response, i.e actin stress fiber formation, upon oxidative stress. In contrast, S pretreatment was shown to reduce thrombin-induced formation of stress fibers in cultured endothelial cells [25], indicating that this drug might influence different signal transduction pathways in parallel, and that the resulting effect on cell morphology is dependent on the cell type and the agonist stimulation.

In human SMCs from LIMA, H_2O_2-induced actin stress fiber formation was not accompanied by an increase in the number of apoptotic cells. However, the potentiation of p38 and HSP27 activation by S pretreatment resulted in apoptosis induction in these cells, indicating that, under certain conditions, activation of SAPK2/p38 may participate in the induction of cell death. In endothelial cells exposed to oxidative stress, membrane blebbing (early signs of apoptosis) could be induced when the extracellular-regulated kinase (ERK) pathway was not appropriately activated in parallel with p38/SAPK2, suggesting that a balance between ERK and SAPK activities was a determining factor in the induction of apoptosis [4]. It was recently shown, in rat cardiac myocytes, that activation of Rac_1 by hypertrophic stimuli could modulate the ERK and/or the JNK cascade, but not the p38/SAPK2 cascade [26]. It is, therefore, possible that, in human SMCs, the blockage by S of the activation of certain prenylated proteins, such as Rac_1, could suppress an important survival function during oxidative stress. The absence of down-regulation of the SAPK2 pathway would then result in apoptosis induction.

The fact that S pretreatment enhances H_2O_2-induced p38 and HSP27 activation could play an important cytoprotective role against oxidative stress in the artery wall. On the other hand, potentiation of the heat shock response by S leads, in our experimental conditions, to an increased number of apoptotic cells. Since a decrease in the number of SMCs has been described mainly at sites of plaque rupture in the atherosclerotic artery, attention should be paid to a potential destabilizing effect of the plaque by S in arteries subjected to oxidative stress.

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