HSP90 inhibition by 17-DMAG attenuates oxidative stress in experimental atherosclerosis

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Aims
Reactive oxygen species (ROS) participate in atherogenesis through different mechanisms including oxidative stress and inflammation. Proteins implicated in both processes, such as mitogen-activated protein kinase kinase (MEK) and some NADPH oxidase (NOX) subunits, are heat shock protein-90 (HSP90) client proteins. In this work, we investigated the antioxidant properties of the HSP90 inhibitor, 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) in experimental atherosclerosis.

Methods and results
Treatment of ApoE−/− mice with 17-DMAG (2 mg/kg every 2 days for 10 weeks) decreased ROS levels and extracellular signal-regulated kinase (ERK) activation in aortic plaques compared with control animals. Accordingly, treatment of rat vascular smooth muscle cells (VSMCs) with 17-DMAG increased HSP27 and HSP70 and inhibited ERK activation. Interestingly, 17-DMAG diminished NADPH oxidase dependent ROS production in VSMCs and monocytes. In addition, a marked reduction in NADPH oxidase dependent ROS production was observed with HSP90siRNA and the opposite pattern with HSP70siRNA. 17-DMAG also diminished the expression of Nox1 and Nox organizer-1 (Noxo1) in VSMCs and monocytes. Interestingly, 17-DMAG was able to modulate ROS-induced monocyte to macrophage differentiation. Finally, higher expression of Nox1 and Noxo1 was found in the inflammatory region of human atherosclerotic plaques, colocalizing with VSMCs, macrophages, and ROS-producing cells.

Conclusion
Our results suggest that HSP90 inhibitors interfere with oxidative stress and modulate experimental atherosclerosis development through reduction in pro-oxidative factors.

Keywords
Atherosclerosis • HSP90 inhibitors • Oxidative stress • Nox1 • Noxo1

1. Introduction
Atherosclerosis is defined as a chronic immune-inflammatory disease caused by subendothelial accumulation of non-native low-density lipoproteins (LDLs), which can be oxidized by reactive oxygen species (ROS) to oxidized LDLs (oxLDLs). In the early stages of atherosclerosis, monocyte-derived macrophages internalize oxLDLs through scavenger receptors such as Cluster of Differentiation 36 (CD36)1 ultimately leading to foam cell formation.2 Migration and deposition of foam cells in the neointima—modulated by CD36 and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent ROS production—are major contributors to plaque development.3 Thus, two processes mediated by ROS, oxidative stress and inflammation,4,5 interact to promote and aggravate atherosclerosis.

ROS are produced by different mechanisms, with NADPH oxidases being a major source of ROS in the cardiovascular system.6,7 Several pro-atherogenic factors such as phorbol-12-myristate-13-acetate (PMA)8 or tumour necrosis factor alpha (TNF-α)9 regulate NADPH activity. Interestingly, a reduction in NADPH oxidase activity is associated with decreased atherosclerotic lesion size.10 NADPH oxidases are membrane-associated multi-enzymes whose classical phagocytic structure is composed of five subunits: the cytosolic factors p67phox, p47phox, and p40phox; and the membrane-associated cytochrome b588, which contains the subunits gp91phox and p22phox.11 Several homologues of the catalytic core gp91phox (Nox2) are found in non-phagocytic cells such as vascular cells12,13 and have been included in the so-called Nox family, which consists of seven homologues (Nox1-5 and Duox1-2). In large vessels, the
predominant NADPH oxidases of vascular smooth muscle cells (VSMCs) are Nox1\textsuperscript{14} and Nox4,\textsuperscript{15,16} both contributing to ROS production. Nox4 is a constitutive enzyme with low catalytic activity, and is involved in physiological signalling. In contrast, Nox1 is inactive in basal conditions,\textsuperscript{17} shows lower expression but high catalytic activity under pathological conditions and is associated with cardiovascular damage.\textsuperscript{18,19} In VSMCs, Nox1 is complexed with p47phox and its homologue Nox organizer-1 (Noxo1). Noxo1 is constitutively active because it lacks the auto-inhibitory motif,\textsuperscript{20} whereas Nox activator-1 (Noxa1) is crucial for Nox1 activation and replaces p67phox in rodents.\textsuperscript{21}

Heat shock protein 90 (HSP90) is a ubiquitous molecular chaperone that ensures the proper conformation of different proteins, including key mediators of signal transduction and transcriptional regulation.\textsuperscript{22} Selective HSP90 inhibitors, such as 17-dimethylaminoethylamine-17-demethoxygeldanamycin (17-DMAG), block the ATP-binding site of HSP90 and exert pleiotropic functions, which include induction of the heat shock response and degradation of some client proteins.\textsuperscript{23} HSP90 inhibitors modulate different signalling pathways including mitogen-activated protein kinases (MAPKs).\textsuperscript{24} The MAPK member mitogen-activated protein kinase kinase (MEK), an HSP90 client protein, is in charge of extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation, a factor that upon activation mediates key processes of VSMCs in atherosclerosis.\textsuperscript{25} We have recently reported that 17-DMAG diminishes the inflammatory response in atherosclerosis through up-regulation of atheroprotective HSPs (e.g. HSP70) and the inhibition of pro-inflammatory transcription factor activity and chemokine expression.\textsuperscript{26} In the present study, we hypothesize that HSP90 inhibition reduces oxidative stress responses in experimental atherosclerosis.

2. Methods

2.1 Reagents

The HSP90 inhibitor 17-DMAG (Biomol) was diluted in saline at 10 mM, stored at −20 °C and was used at a final concentration of 200 nM.\textsuperscript{26} TNF-α was purchased from Preprotech and used at 25 ng/mL.\textsuperscript{27} The NADPH oxidase inhibitor apocynin was added to the cultures for 1 h, at a final concentration of 100 μM.\textsuperscript{28} PMA was used at 100 nM.\textsuperscript{29} Unless otherwise specified, all other chemicals were purchased from Sigma-Aldrich.

2.2 Experimental atherosclerosis

Twelve-week-old male ApoE\textsuperscript{−/−} mice (Jackson Laboratory) were fed on a Western diet \citep{21.2%fat} and was used at a final concentration of 200 nM.\textsuperscript{26} TNF-α was purchased from Preprotech and used at 25 ng/mL.\textsuperscript{27} The NADPH oxidase inhibitor apocynin was added to the cultures for 1 h, at a final concentration of 100 μM.\textsuperscript{28} PMA was used at 100 nM.\textsuperscript{29} Unless otherwise specified, all other chemicals were purchased from Sigma-Aldrich.

2.3 Cell culture

Male Wistar rats were euthanized with 200 mg/kg of pentobarbital sodium, their aortas were removed, and VSMCs were isolated and maintained in DMEM (BioWhittaker) supplemented with 10% foetal bovine serum (FBS, BioWhittaker), 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). Cells were used between passages 3 and 7. A human THP-1 monocyte cell line was purchased from ATCC (CRL-1593) and cultured in RPMI 1640 (BioWhittaker) supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. For in vitro experiments in VSMCs, cells were made quiescent by incubation in medium without FBS for 24 h.

2.4 Detection of superoxide anion

Dihydroethidium (DHE; Molecular Probes, Invitrogen) was used to evaluate in situ levels of superoxide as described previously\textsuperscript{30} (see Supplementary material online).

2.5 Transfection of small-interfering RNA

100 μg/mL streptomycin. For in vitro experiments in VSMCs, cells were made quiescent by incubation in medium without FBS for 24 h. The treated

2.6 Patients

Ten atherosclerotic plaques (stages V–VI) from patients undergoing carotid endarterectomy in our institutions were fixed with paraformaldehyde and embedded in paraffin for histological analysis. In addition, 10 carotid endarterectomy samples were dissected by a trained vascular surgeon separating the stenosing culprit plaque (CP) from the non-complicated (NCP) fibrous adjacent area as previously described.\textsuperscript{31} The CP was defined as the lesion, usually localized at the origin of the internal carotid artery responsible for the surgery. The CP contained an important proportion of inflammatory cells (Stary stages V–VI), whereas the NCP adjacent areas were mainly composed of VSMCs and lipid deposits (Stary stage III). The study was approved by the hospital’s ethics committee (IIS-Fundación Jiménez Díaz) according to the institutional and the Good Clinical Practice guidelines, which was performed in accordance with the Declaration of Helsinki. All participants gave written informed consent.

2.7 Immunohistochemistry

O.C.T.TM (Sakura)-embedded samples from mice arteries were incubated with rabbit polyclonal anti-pERK1/2 (sc-16981-R, Santa Cruz Biotechnologies), followed by rabbit secondary antibody, ABCComplex/HRP (Vector laboratories) was added, sections were stained with 3,3’-diaminobenzidine (Dako) and mounted in Pertex (Histolab). Once immunohistochemistry had been carried out, data were collected using a Nikon Eclipse D400 microscope using bright field imaging at ×20 with NIKON ACT-1 software. Quantification of positive staining was performed using Image Pro Plus®; the proven SolutionTM version 4.5. Positive staining in the regions of interest was computed with the use of positive colour intensity thresholds. Quantification was performed by a pathologist, blind to the experimental groups analysed. Immunohistochemistry data are expressed as a percentage of the positive immunostained area vs. the total area as described.\textsuperscript{30}

Paraffin-embedded human carotid atherosclerotic plaques were cross-sectioned into 4 μm thick pieces, dewaxed, and rehydrated. For colocalization studies in human plaques, double immunohistochemistry/immuno-fluorescence with rabbit polyclonal anti-Nox1 (sc-25545, Santa Cruz Biotechnologies) and Noxo1 (AP09301PU-N, Acris Antibodies GmbH) was carried out in the same sections followed by either α-actin (clone 1A4, M0851, Dako) or CD68 (clone kp1, M0814, Dako) staining, as previously described.\textsuperscript{31} Following ABCComplex/HRP for Nox1 or Noxo1, sections were stained with 3-amoio-9-ethycarbazole plus high-sensitivity
The lucigenin-enhanced chemiluminescence assay was used to determine ROS production. Data obtained after treatment are expressed as the fold change relative to the untreated control.

2.11 Measurement of NADPH activity
The lucigenin-enhanced chemiluminescence assay was used to determine the NADPH oxidase activity in cell homogenates as previously described. Briefly, cells were lysed in buffer-containing protease inhibitors (1% protease inhibitor cocktail) and subjected to Dounce homogenization (100 times, on ice), and the homogenate was stored until use. The reaction mixture comprised 50 mM phosphate buffer, 0.01 mM EDTA, 0.32 M sucrose, 5 μM lucigenin, and 0.1 mM NADPH, in a total volume of 500 μL. A low concentration of lucigenin (5 μM) was used to avoid auto-oxidation. The reaction was started by the addition of lucigenin to the cell homogenate (50 μg of protein) and the light emission was recorded every 10 s for 5 min in a tube luminometer Sirius (Berthold Detection System). There was no measurable activity in the absence of NADPH. The chemiluminescence, which was measured for 5 min after the addition of NADPH, was recorded in a luminometer Sirius (Berthold Detection System). No activity could be measured in the absence of NADPH. The ROS production was determined from the ratio of the relative light units to the total protein levels and expressed as fold vs. basal.

2.12 Statistical analysis
In vitro experiments were performed at least three times. Results are expressed as mean ± SEM and were analysed by the Mann–Whitney non-parametric or Student’s t-test when appropriate. The Wilcoxon paired test was used to analyse the differences between NCP and CP from the same patients. Univariate association between HSP70 and DHE staining in mice atherosclerotic plaques was performed by Spearman’s correlation test. Statistics were performed using GraphPAD InStat (GraphPAD Software). Differences were considered significant with P < 0.05.

3. Results

3.1 HSP90 inhibition by 17-DMAG diminishes ROS production and ERK activation in vivo
We previously showed that in vivo treatment with 17-DMAG attenuated lesion size, lipid content, and macrophage infiltration in atherosclerotic plaques of ApoE−/− mice. Using the same animal model, we found a significant decrease in superoxide levels in the 17-DMAG group compared with the vehicle-injected group (Figure 1A and B). Since we previously observed that HSP70 was increased in the 17-DMAG injected group, we analysed the possible relationship between DHE staining and HSP70 expression in the atherosclerotic plaques. Analysis of serial sections from mice aortic plaques revealed a significant negative correlation between DHE staining and HSP70 expression (r = −0.5; P < 0.001).

Furthermore, since ERK activation is involved in ROS production, and the upstream kinase MEK is an HSP90 client protein, we studied the effect of 17-DMAG treatment on ERK phosphorylation in mice atherosclerotic plaques. We found decreased ERK 1/2 phosphorylation in the 17-DMAG-treated group compared with vehicle-injected animals (Figure 1C and D).

3.2 Pleiotropic effects of 17-DMAG in VSMCs
The effects of HSP90 inhibition by 17-DMAG include the induction of some members of the HSP family and the modulation of several signalling pathways. We assessed HSP27 and HSP70 expression under basal and pro-oxidative conditions. We found an increase—at the doses and times studied (data not shown)—at the protein and mRNA levels—in HSP27 and HSP70 induced by 17-DMAG but not by TNF-α treatment (Supplementary material online, Figure S1). No cytotoxic effects of 17-DMAG were observed at the doses and times studied (data not shown).

Next, we examined the effect of this HSP90 inhibitor on ERK1/2 activation. We show that treatment with 17-DMAG diminishes basal ERK1/2 phosphorylation at 4 and 8 h (Figure 2A and B). To further evaluate the effects of 17-DMAG on ERK1/2 under a pro-oxidative scenario, we stimulated VSMCs with TNF-α. This pro-oxidative cytokine rapidly increased ERK1/2 phosphorylation, an effect abolished by the HSP90 inhibitor pretreatment (Figure 2C and D).
### 3.3 17-DMAG decreases ROS production and NADPH oxidase activity in VSMCs

To analyse the mechanisms involved in the modulation of oxidative stress by 17-DMAG in vivo, cultured VSMCs were incubated with TNF-α in the absence or presence of 17-DMAG. Exposure to TNF-α augmented ROS levels in VSMCs, which was prevented by pre-treatment with 17-DMAG, while 17-DMAG alone did not significantly modify basal ROS levels (Figure 3A and B). The potential involvement of NADPH oxidase in the ROS production by VSMCs was confirmed by the use of the inhibitor apocynin, which abolished the pro-oxidative effect of TNF-α (Figure 3B). Furthermore, pretreatment with 17-DMAG inhibited the NADPH oxidase activity induced by TNF-α in VSMCs (Figure 3C). Interestingly, 17-DMAG was also able to decrease the basal levels of NADPH oxidase activity (Figure 3C). To study the specific effect of HSP90 inhibition, we measured basal NADPH-dependent ROS production under genetic silencing of either HSP90 or HSP70 (Supplementary material online, Figure S2). We found that genetic inhibition of HSP90 abolished ROS production while HSP70 silencing resulted in a marked increase (Figure 3D).

### 3.4 17-DMAG attenuates NADPH oxidase subunit expression

HSP90 is a chaperone that interacts with multiple client proteins, among them some NADPH subunits. Thus, we examined the expression of major NADPH subunits in VSMCs such as Nox1, Noxo1, p22phox, Nox4, and Noxa1 and the potential modulation...
by 17-DMAG. In basal conditions, HSP90 inhibition by treatment with 17-DMAG diminished Nox1 and Noxo1 mRNA levels (Figure 4A). Furthermore, incubation of cells with TNF-α for 4 h gave rise to an increase in the catalytic NADPH oxidase subunit Nox1 and the regulatory subunit Noxo1 at both the protein and mRNA level (Figure 4B and C). The induction of Nox1 and Noxo1 expression was prevented by pretreatment with 17-DMAG (Figure 4B and C). In contrast, no significant effects of TNF-α were observed on Nox4, p22phox, and Noxa1 mRNA expression at the dose and times studied (Figure 4D).

Figure 4 Effect of HSP90 inhibition on NADPH subunit expression in rat VSMCs. (A) Nox1 and Noxo1 mRNA quantification by real-time PCR in VSMCs treated with 17-DMAG (200 nM, at the indicated times). (B) Representative immunoblots of Nox1, Noxo1, and β-actin protein in VSMCs treated with TNF-α (25 ng/mL, 4 h) in the absence or presence of 17-DMAG (200 nM, 4 h pre-incubation). (C) Nox1 and Noxo1 mRNA quantification by real-time PCR in VSMCs treated with TNF-α (25 ng/mL, 4 h) in the absence or presence of 17-DMAG (200 nM, 4 h pre-incubation). (D) mRNA quantification of different NADPH oxidase subunits by real-time PCR of VSMCs treated with TNF-α (25 ng/mL, 4 h) in the absence or presence of 17-DMAG (200 nM, 4 h pre-incubation). Values shown are means ± SEM of three independent experiments.

Figure 3 ROS production in rat VSMCs. (A) Representative DHE staining of rat VSMCs under basal conditions and stimulated with TNF-α (25 ng/mL, 10 min) in the presence or absence of 17-DMAG (200 nM, 4 h pre-incubation). (B) Quantification of ROS production by DHE staining in rat VSMCs [TNF-α (25 ng/mL, 10 min), 17-DMAG (200 nM, 4 h pre-incubation), apocynin (100 μM, 1 h pre-incubation)]. (C) NADPH oxidase activity in rat VSMCs stimulated with or without TNF-α (25 ng/mL, 10 min), and in the absence or presence of 17-DMAG (200 nM, 4 h pre-incubation). (D) NADPH-dependent ROS production in rat VSMCs transfected with vehicle (−), scrambled (scr), and specific siRNA for HSP90α/β (si90) and HSP70 (si70) (30 nM, 24 h for all). Values shown are means ± SEM of three independent experiments.

by 17-DMAG. In basal conditions, HSP90 inhibition by treatment with 17-DMAG diminished Nox1 and Noxo1 mRNA levels (Figure 4A). Furthermore, incubation of cells with TNF-α for 4 h gave rise to an increase in the catalytic NADPH oxidase subunit Nox1 and the regulatory subunit Noxo1 at both the protein and mRNA level (Figure 4B and C). The induction of Nox1 and Noxo1 expression was prevented by pretreatment with 17-DMAG (Figure 4B and C). In contrast, no significant effects of TNF-α were observed on Nox4, p22phox, and Noxa1 mRNA expression at the dose and times studied (Figure 4D).
3.5 17-DMAG prevents NADPH oxidase-dependent ROS production and macrophage differentiation in vitro

To study the effects of HSP90 inhibition on other relevant cells present in the atherosclerotic plaque, parallel studies were performed in monocytes (THP-1 human cell line). PMA induced NADPH-dependent ROS production (Figure 5A) and Nox1 and Noxo1 expression (Figure 5B) in a time-dependent manner in THP-1 cells, which was significantly reduced by pretreatment with 17-DMAG. Similar to VSMCs, basal NADPH oxidase-dependent ROS generation and Nox1 and Noxo1 levels were also reduced by 17-DMAG in monocytes. Interestingly, 17-DMAG prevented the cell morphology changes associated with monocyte-to-macrophage differentiation by PMA in our experimental conditions (Figure 5C). This was further verified by analysing CD36 expression, a macrophage differentiation marker. PMA time-dependently induced CD36 mRNA, which was diminished by 17-DMAG (Figure 5D).

3.6 Nox1 and Noxo1 are increased in the culprit plaques of human atherosclerotic plaques

To elucidate the potential relevance of our studies in human atherosclerosis, we examined the expression of Nox1 and Noxo1 proteins in human carotid atherosclerotic plaques. We found that Nox1 and Noxo1 staining was mainly localized in the inflammatory region of the shoulder, characterized by a high macrophage accumulation, although it was also present in the fibrous region, with increased VSMCs and collagen content (Figure 6A and B). Colocalization studies confirmed that both Nox1 and Noxo1 are expressed by CD68 and α-actin positive cells in advanced human carotid atherosclerotic plaques (Supplementary material online, Figure S3A and B).

In addition, we found colocalization of Nox1 and Noxo1 with DHE positive cells (Supplementary material online, Figure S3C). Lastly, we quantified Nox1 and Noxo1 levels by western blotting in tissue extracts from complicated areas (CP, containing an important proportion of inflammatory cells, Stary stages V–VI) and the adjacent non-complicated region (NCP, mainly composed of VSMCs and lipid deposits, Stary stage III) of the plaques. Western blot analysis revealed a significant increase in both NADPH subunits in the CP region of human advanced atherosclerotic plaques when compared with the NCP areas (Figure 6C and D).

4. Discussion

Since ROS are a common feature of the ‘vicious circle’ of oxidative stress and inflammation in the arterial wall, therapies to prevent the pathological augmentation of ROS production in the vascular system are of interest. We found that treatment with the HSP90 inhibitor 17-DMAG significantly reduced ROS levels in atherosclerotic plaques of ApoE−/− mice when compared with controls. Moreover, in vitro experiments showed analogous results in VSMCs and monocyte-derived macrophages, as 17-DMAG partially reversed the TNF-α or PMA-induced ROS production.

The pleiotropic effects of HSP90 inhibition by 17-DMAG include induction of HSP synthesis and degradation of HSP90 client proteins, thereby modulating several signalling pathways. On the one hand, we show that pretreatment with the HSP90 inhibitor 17-DMAG up-regulates HSP27 and HSP70 mRNA and protein levels in VSMCs. Interestingly, we found an inverse correlation between HSP70 expression and DHE staining in the atherosclerotic plaques from ApoE−/− mice. Accordingly, we found that HSP70 knockdown markedly increased ROS generation in VSMCs. In this context,
previous studies have shown that overexpression of HSP70 exerts anti-oxidative effects in a rat model of atherosclerosis. Furthermore, the anti-oxidant effects of resveratrol treatment in atherosclerosis are accompanied by a dose-dependent augmentation of HSP27. On the other hand, the pleiotropic effects of HSP90 inhibition include the modulation of several signalling pathways such as the MAPK cascade, in particular ERK1/2, a well-known redox-sensitive kinase. Accordingly, mice treated with 17-DMAG showed decreased levels of ERK1/2 phosphorylation in atherosclerotic plaques. This effect was corroborated by in vitro experiments in which stimulation of VSMCs with 17-DMAG was able to reverse both basal and TNF-α-induced ERK1/2 phosphorylation. In agreement, previous studies showed that HSP90 inhibition using 17-AAG or 17-DMAG diminishes ERK phosphorylation in different cell types. Thus, up-regulation of atheroprotective HSPs and attenuation of ERK activation by 17-DMAG could inhibit oxidative stress in atherosclerosis.

Interestingly, 17-DMAG was also able to reduce both basal and TNF-α-induced NADPH oxidase activity, a main source of ROS in vascular cells. Since the NADPH catalytic subunit Nox1 and HSP90 interact through the C-terminal residue of Nox1, it is tempting to speculate that the 17-DMAG/HSP90 binding prevents Nox1/HSP90 interaction. In this respect, we have observed that HSP90 inhibition by HSP90α/β siRNA clearly inhibited basal NADPH-dependent ROS production in VSMCs. Moreover, we found that 17-DMAG was able to reduce both the basal and the TNF-α-induced expression of Nox1 and Noxo1, but not the mRNA levels of other NADPH subunits analysed. In agreement with Chen et al., the reduced levels in Nox subunits following HSP90 inhibition is not necessarily related to NADPH oxidase activity reduction, but it is another effect of HSP90 inhibition that could be atheroprotective during long-term treatment. In the present work, we demonstrate that 17-DMAG can effectively inhibit PMA-induced NADPH oxidase-dependent ROS production, Nox1 and Noxo1 overexpression, together with CD36, a hallmark of monocyte-to-macrophage differentiation. Thus, the long-term functional consequences of HSP90 inhibition by 17-DMAG could include decreasing NADPH-dependent ROS production involved in macrophage differentiation, a key process implicated in foam cell retention and atherogenesis.

To clarify the relevance of Nox1 and Noxo1 modulation in atherosclerosis, we analysed the expression of both proteins in human carotid atherosclerotic plaques. Although Nox1 mRNA levels have been previously analysed in human atherosclerotic plaques, we are not aware of any studies that have addressed Nox1 or Noxo1 protein levels in human atherosclerotic plaques. We first performed an observational study in which we found that Nox1 and Noxo1 staining was mainly localized in the inflammatory region of the shoulder, characterized by high macropage accumulation, although it was also present in the fibrous region. Moreover, we show Nox1 and Noxo1 expression not only in macrophages, but also in VSMCs of human atherosclerotic plaques. Besides, we found colocalization between both subunits and DHE positive cells, which suggests that cells expressing high levels of Nox1 and Noxo1 produce elevated levels of ROS. We further quantified and confirmed an increased protein expression of Nox1 and Noxo1 in the culprit region of the human atherosclerotic plaques compared with their respective non-complicated region. These data highlight the potential importance of Nox1-derived ROS in the chronic immune-inflammatory oxidative scenario present in human advanced atherosclerotic lesions.

In summary, we have shown that 17-DMAG is able to reduce oxidative stress in experimental atherosclerosis. Although further studies are needed to clarify the role of HSP90 as a possible therapeutic target in atherosclerosis, its present use in clinical trials should facilitate the evaluation of HSP90 inhibitors in non-neoplastic disorders, such as cardiovascular diseases.

Supplementary material
Supplementary material is available at Cardiovascular Research online.

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