Preconditioning by Mitochondrial Reactive Oxygen Species Improves the Proangiogenic Potential of Adipose-Derived Cells–Based Therapy

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Objective—Transplantation of adipose-derived stroma cells (ADSCs) stimulates neovascularization after experimental ischemic injury. ADSC proangiogenic potential is likely mediated by their ability to differentiate into endothelial cells and produce a wide array of angiogenic and antiapoptotic factors. Mitochondrial reactive oxygen species (ROS) have been shown to control ADSC differentiation. We therefore hypothesized that mitochondrial ROS production may change the ADSC proangiogenic properties.

Methods and Results—The use of pharmacological strategies (mitochondrial inhibitors, antimycin, and rotenone, with or without antioxidants) allowed us to specifically and precisely modulate mitochondrial ROS generation in ADSCs. We showed that transient stimulation of mitochondrial ROS generation in ADSCs before their injection in ischemic hindlimb strongly improved revascularization and the number of ADSC-derived CD31-positive cells in ischemic area. Mitochondrial ROS generation increased the secretion of the proangiogenic and antiapoptotic factors, VEGF and HGF, but did not affect ADSC ability to differentiate into endothelial cells, in vitro. Moreover, mitochondrial ROS-induced ADSC preconditioning greatly protect ADSCs against oxidative stress–induced cell death.

Conclusion—Our study demonstrates that in vitro preconditioning by moderate mitochondrial ROS generation strongly increases in vivo ADSC proangiogenic properties and emphasizes the crucial role of mitochondrial ROS in ADSC fate.
(*Arterioscler Thromb Vasc Biol*. 2009;29:1093-1099.)

Key Words: adipose tissue ■ stem cells ■ angiogenesis ■ oxidative stress ■ mitochondria

Cardiovascular diseases, as well as cardiac ischemia and lower limb vascularization, are associated with obesity and type 2 diabetes and constitute a major public health problem. The ability of the organism to form a collateral network of blood vessels determines to a large part the clinical consequences and severity of tissue ischemia. The development of new vessels is significantly reduced in diabetic patients with peripheral artery disease.[1,2] This contributes to the severe course of limb ischemia in diabetic patients, which often results in foot ulceration and lower extremity amputation. Recent advances in understanding stem cell biology have prompted the initiation of clinical trials of vascular cell therapy. Although significant and encouraging results have been obtained from studies in which human bone marrow or circulating blood cells have been used, obtaining sufficient numbers of these cells is a major constraint.[3] Recent studies have identified adipose tissue as a new source of stem cells, some of which may be suitable for the restoration of vascular network and function.[4,5,6]

The close interaction between adipogenesis and vasculogenesis appears as early as during embryogenesis, where the formation of capillary convolutions is a decisive and specific phase of fat lobules development.[7,8,9] Later, adipose tissue remodeling and functioning as a metabolic and endocrine tissue require development of the capillary network. This interaction between adipogenesis and blood vessel growth is consistent with recent data describing that antiangiogenic agents induce adipose tissue loss or prevents diet-induced and genetic obesity in mice.[10,11] This close relationship is reinforced by the ability of adipose lineage cells to produce potent proangiogenic factors such as monobutyril, vascular endothelial growth factor (VEGF), and leptin.[12–15] Recently, several evidences point to a great potential for adipose-derived stroma cell (ADSC)-based therapy to promote neo-

Received August 7, 2007; revision accepted April 23, 2009.
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***Arterioscler Thromb Vasc Biol* is available at http://atvb.ahajournals.org** **DOI: 10.1161/ATVBAHA.109.188318**

vascularization: (1) the expression of CD34 by both murine and human cultured ADSCs,[10] (2) the ability of cultured ADSCs from mice and human origin to form capillary structures and express VonWillebrand antigen in semisolid medium,[4] (3) the great enhancement of neovascularization in ischemic tissues by transplantation of ADSCs through their ability to differentiate into endothelial cells and produce angiogenic and antiapoptotic factors.[4–6]

It is likely that ADSCs are bipotent progenitor cells and can be considered both as preadipocytes and vascular progenitors.[4,16] The pathways that modulate these plastic processes are as yet unknown. However, one can reasonably speculate that inhibition of ADSC differentiation into adipocytes may improve their proangiogenic potential and their ability to differentiate into endothelial cells, this being of a great interest in understanding fat development as well as in a therapeutic point of view.

Reactive oxygen species (ROS) and reactive nitrogen species are biologically active O_2 derivatives, first considered as toxic molecules but now recognized as very important physiological signaling molecules.[17] Interestingly, ROS have been shown to play major and positive roles in blood vessel growth as well as in vivo preconditioning protection.[18] Although ROS can originate from different subcellular sources, mitochondrial ROS are considered as the main source of ROS, ie, 80% of anion superoxide is provided by the respiratory chain. Various reports indicate that mitochondrial ROS act as true second messenger in the O_2 sensing as well as nutrient-sensing mechanisms of several cells.[19–22] We recently demonstrated that mitochondrial ROS negatively control adipose tissue development by inhibiting both ADSC proliferation and differentiation into adipocytes via the adipogenic repressor CHOP-10/GADD153.[23,24] We therefore hypothesized that mitochondrial ROS may also modulate the proangiogenic potential of ADSCs.

Using pharmacological strategies to finely tune a moderate mitochondrial ROS generation, we demonstrate herein that mitochondrial ROS preconditioning of cultured ADSCs greatly enhances their proangiogenic properties.

Methods

Chemicals

Antimycin, rotenone, pyrrolidine dithiocarbamate (PDTC), and N-Acetyl Cystein (NAC) were purchased from Sigma.

Animals and Tissue Sampling

Seven-week-old male C57Bl/6 (Charles River, France) mice were housed under 12 hours light/dark cycle at 21°C with free access to water and a standard chow diet. They were killed by cervical dislocation under CO_2 anesthesia. Inguinal white adipose tissue was dissected immediately after sacrifice. The experiments were performed in accordance with the European Community guidelines for the care and use of laboratory animals (EEC/No. 07430). GFP mice were kindly provided by Dr Okabe (Genome Information Research Center, Osaka University, Japan).[25]

ADSC Isolation and Culture

ADSCs were prepared as previously described[4] and seeded at 10 000 cells/cm^2 in DMEM-F12 10% NCS medium.

Pharmacological Treatments

Four days after seeding, ADSCs were incubated with antimycin (40 nmol/L) or rotenone (10 nmol/L) for 48 hours. Antioxidants NAC (0.3 mmol/L) or PDTC (500 nmol/L) were added 18 hours before addition of antimycin or rotenone.

Determination of Intracellular ROS Generation

Intracellular ROS generation was assessed using 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate, diacetoxymethylester (H_2-DCFDA) as previously described.[24]

Model of Hindlimb Ischemia

Mice underwent surgical ligation of the proximal part of the right femoral artery as previously described.[26] Five hours later, 1.10^6 cells were administered by intramuscular injection in 3 different sites (gastrocnemius, gracilis, and quadriceps muscles, respectively, 25 μL per injection) of the ischemic leg, as previously described[4] and vessel density was evaluated as previously described.[4,26]

Adipocyte Differentiation Assessment

After 4 days of culture, medium was replaced by DMEM-F12 10% NCS supplemented with 875 nmol/L insulin, 33 nmol/L dexamethasone, 10 μg/mL transferring, and 2 nmol/L T3. ADSCs were transiently treated with antimycin for 48 hours and adipocyte differentiation was assessed 2 days later by the measurement of triglyceride content (Triglycerides enzymatique PAP 150, Biomerieux). For visualization of differentiated adipocytes, cells were stained with Oil Red O.

Apoptosis and Necrosis

Medium of ADSCs treated for 48 hours with antimycin or antioxidant was replaced by DMEM-F12 without NCS and supplemented with 75 μmol/L hydrogen peroxide. Necrosis and apoptosis were then evaluated concomitantly. The first technique evaluated apoptosis and necrosis on intact cultured cells after fluorescent staining by using 2 fluorescent dyes: 0.6 μmol/L SYTO-13 and 15 μmol/L propidium iodide.[27] In the second technique, necrosis and apoptosis were evaluated with Annexin V-FITC apoptosis detection kit (Calbiochem) according to the RAPID protocol of the manufacturer instruction.

Determination of HGF and VEGF Protein Levels

VEGF and HGF assays (RIA, R&D systems) were performed on the collected supernatant of ADSCs treated for 48 hours with or without antimycin and/or antioxidant.

Cell Phenotyping

After 48 hours of treatment with antimycin, ADSCs were labeled with anti–CD31-PE and anti–CD144-FITC or with anti–Flk1-PE and analyzed on a fluorescence-activated cell sorter. All the antibodies were purchased from BD Biosciences, except CD144 which was obtained from Serotec.

Statistical Analysis

Experiments were repeated at least 3 times. Quantitative results were expressed as mean±SEM. Student *t* test or Mann–Whitney *U* test were used for statistical analysis of in vitro experiments. For in vivo experiments, 1-way analysis of variance ANOVA was used to compare each parameter. Post-hoc Bonferonni *t* test comparisons were then performed to identify which group differences account for the significant overall ANOVA. Values of $P<0.05$ were considered significant.

Results

Antimycin-Induced Mitochondrial ROS Overproduction

To quantitatively and finely modulate mitochondrial ROS generation, we chose pharmacological approaches, as previously described on 3T3-F442A preadipocyte cell line.[24] First,

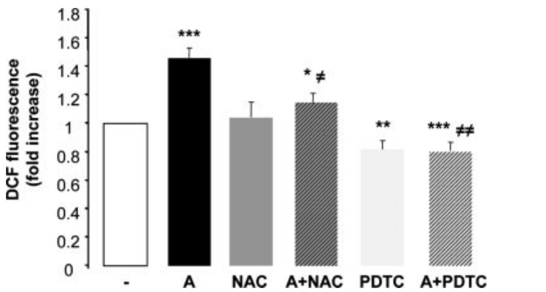

Figure 1. Antimycin increases mitochondrial ROS generation. ADSCs were incubated for 1 hour with antimycin (A) with or without antioxidant NAC or PDTC. Control ADSCs were treated with ethanol (−). Quantitative evaluation of ROS generation using H_2-DCFDA probe is shown. n=3. *$P<0.05$, **$P<0.01$, and ***$P<0.001$ vs control ADSCs; ≠$P<0.05$ and ≠≠$P<0.01$ vs antimycin-treated ADSCs.

we used antimycin (A), a well-known specific inhibitor of mitochondrial complex III[28] at a concentration of 40 nmol/L, which induces only moderate inhibition of cell respiration (around 20% of respiration, data not shown). At this moderate concentration, antimycin stimulated ROS production as revealed by a significant increase in DCF fluorescence in ADSCs (1.46±0.07% compared to untreated control cells, $P<0.001$, Figure 1). We analyzed the effects of additional presence of different antioxidants, NAC and PDTC, on antimycin-induced mitochondrial ROS generation. We carefully selected concentrations of antioxidants, which had no significant or poor effect per se on basal ROS generation. Antimycin-induced ROS overproduction was significantly prevented whatever the antioxidant treatments (Figure 1). The use of MitoSOX, a probe specific to mitochondrial ROS, clearly confirmed that antimycin treatment generated a mitochondrial oxidative stress (supplemental Figure I, available online at http://atvb.ahajournals.org). Altogether, our results demonstrate that these pharmacological treatments specifically affect mitochondrial ROS generation in ADSCs.

In Vitro Preconditioning by Mitochondrial ROS Generation Enhances Proangiogenic Potential of ADSCs In Vivo

We next investigated the effect of these treatments on proangiogenic potential of ADSCs in vivo. Transplantation of control ADSCs in hindlimb after ischemia is the most powerful situation to reveal their proangiogenic potential. As previously reported,[4] angiography scores, capillary density, and foot perfusion were significantly increased by 1.6- ($P<0.01$, Figure 2A), 1.4- ($P<0.05$, Figure 2C), and 1.5-fold ($P<0.01$, Figure 2E) respectively, in mice receiving ADSCs compared to PBS-injected animals. In vitro pretreatment of ADSCs with antimycin for 48 hours further significantly enhanced their proangiogenic potential (Figure 2A through 2F). Injection of antimycin alone in injured leg did not affect postischemic vessel growth (data not shown). In vitro cotreatment with antioxidants NAC or PDTC fully abrogated the antimycin-induced increase of ADSC proangiogenic potential (Figure 2A thorugh 2F). It is noteworthy that antioxidants alone did not change ADSC angiogenic potential compared to untreated cells (data not shown).

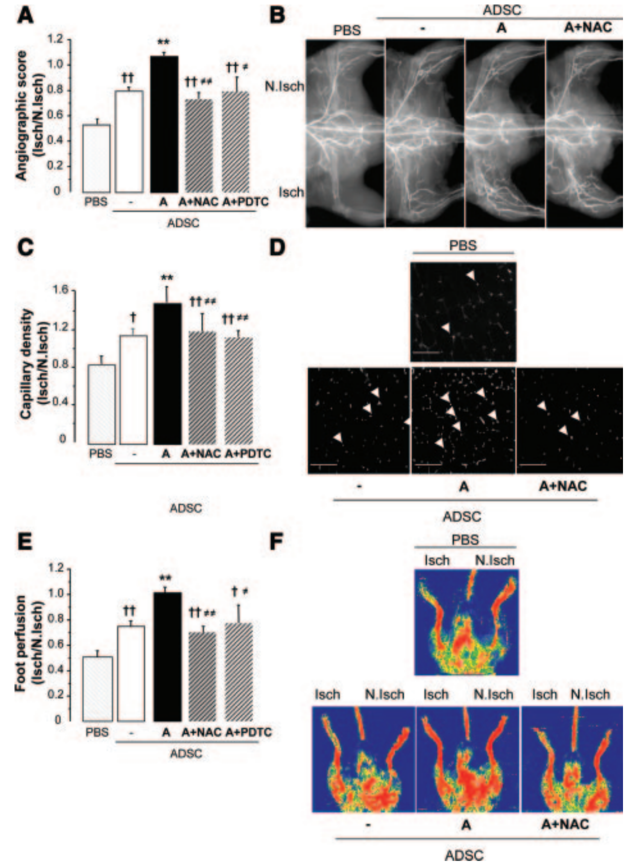

Figure 2. In vitro exposure to mitochondrial ROS generated by antimycin enhances in vivo proangiogenic properties of ADSCs. After in vitro pretreatment, 1.10^6 cells were intramuscularly injected in the ischemic hindlimb for 14 days. Quantitative evaluation and representative photomicrographs of microangiography (A and B), capillary density (C and D), and foot perfusion (E and F) in ischemic mice are shown. PBS means mice injected with PBS only. n=8. †$P<0.05$ and ††$P<0.01$ vs PBS injected mice; **$P<0.01$ vs mice receiving control ADSCs; ≠$P<0.05$ and ≠≠$P<0.01$ vs mice receiving antimycin-treated ADSCs. Each bar represents 100 μm.

To further demonstrate the role of mitochondrial ROS, we performed similar experiments in vitro as well as in vivo using rotenone, another mitochondrial electron transport inhibitor able to promote mitochondrial ROS generation but by a different mechanism than antimycin, ie, via the complex I.[23,24] Similarly to antimycin, rotenone (at a moderate concentration that inhibits respiration of around 20%) increased mitochondrial ROS generation, and this increase was prevented by NAC cotreatment (Figure 3A). Furthermore, in vitro pretreatment of ADSCs with rotenone enhanced ADSC proangiogenic properties, as shown by significant increase of angiographic score, capillary density, and foot perfusion (Figure 3B). All these effects were abolished by antioxidant treatment.

Altogether, these results demonstrated that ADSC preconditioning by moderate and transient mitochondrial ROS generation significantly enhanced ADSC proangiogenic properties. Three equally tenable and nonexclusive hypotheses regarding the origin mechanisms underlying activation of ADSC proangiogenic properties by mitochondrial ROS could be suspected: (1) activation of ADSC differentiation into endothelial lineage rather than adipocyte one, (2) increase in

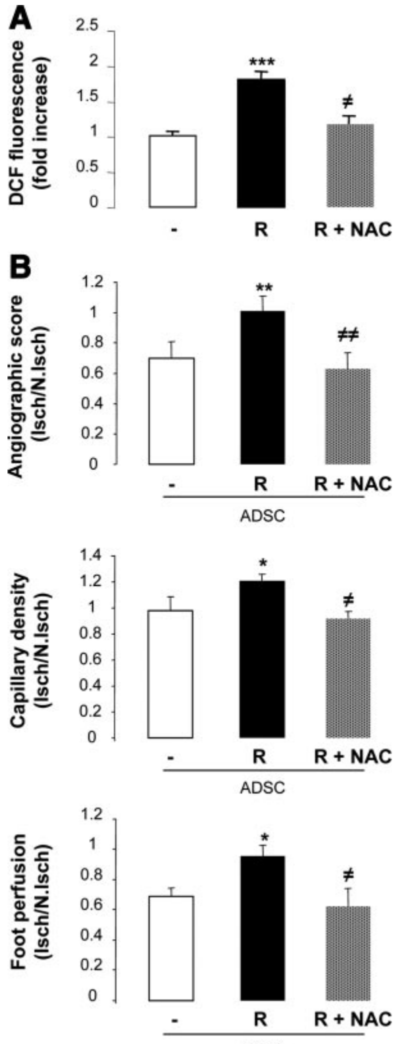

Figure 3. In vitro exposure to mitochondrial ROS generated by rotenone enhances in vivo proangiogenic properties of ADSCs. After in vitro pretreatment, quantitative evaluation of ROS generation (A), microangiography, capillary density, and foot perfusion (B) were performed. n=8. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs control cells; ≠$P<0.05$, ≠≠$P<0.01$ vs rotenone-treated ADSCs.

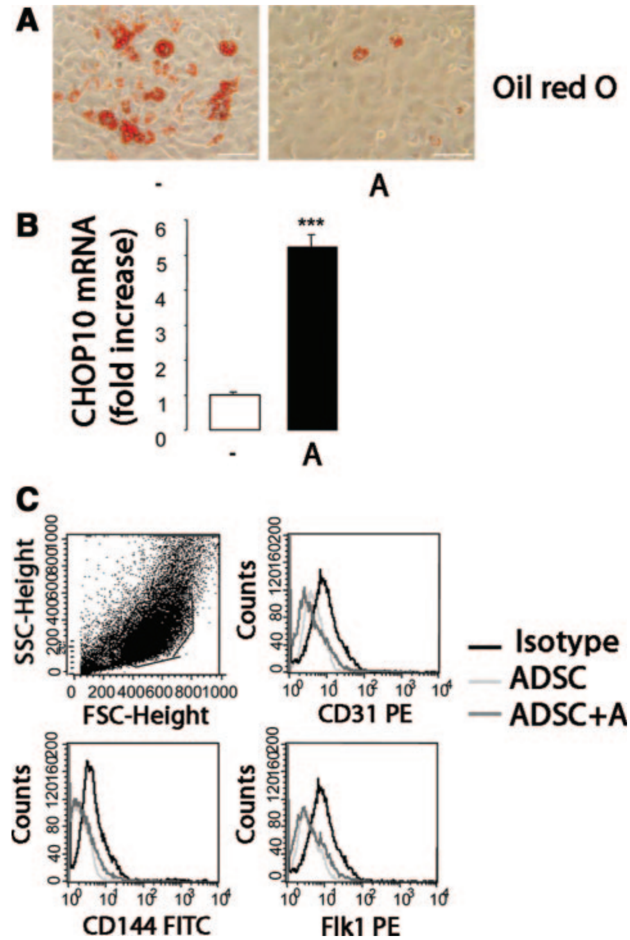

Figure 4. Mitochondrial ROS effects on in vitro differentiation of ADSCs. A, Two days after antimycin treatment, adipocyte differentiation was assessed by Oil red O staining. B, CHOP-10/GADD153 mRNA levels were quantified by real-time PCR analysis. C, FACS analyses were performed to detect expression of endothelial markers, CD31, Flk1, and CD144. n=4. ***$P<0.001$ vs control ADSCs. Each bar represents 60 μm.

release of angiogenic or antiapoptotic factors, and (3) improved ADSC survival in ischemic areas. We then successively investigated these different points.

Mitochondrial ROS Effects on Endothelial Differentiation and Paracrine Secretions of ADSCs

In vitro mitochondrial ROS preconditioning inhibited adipocyte differentiation but did not induce expression of endothelial cell markers on ADSCs.

The increase of neovascularization by mitochondrial ROS pretreatment could be the consequence of the preferential differentiation of ADSCs toward the endothelial lineage instead of their differentiation toward adipocytes. As early demonstrated on preadipocyte cell line 3T3-F442A,[24] antimycin greatly inhibited adipocyte differentiation of ADSCs, as shown by reduction in triglyceride contents (59.6±9 in antimycin-treated ADSCs compared to 129.3±10.6 μg triglycerides/mg proteins in control ADSCs, $P<0.01$) and Oil Red O staining (Figure 4A). Both NAC and PDTC significantly abrogate antimycin-induced inhibition of ADSC differentiation into adipocyte as previously observed in 3T3-F442A cell line[24] (data not shown). Moreover, as demonstrated in 3T3-F442A cells, the antimycin-related inhibition of adipocyte differentiation was strongly correlated with a high increase in the adipogenic repressor CHOP-10/GADD153 expression (Figure 4B). FACS analysis presented in Figure 4C shows that control ADSCs in liquid medium did not express classic mature endothelial markers such as CD31, CD144, and Flk1. Moreover, no change was observed after in vitro antimycin treatment.

Therefore, antimycin treatment for 48 hours was able to inhibit adipocyte differentiation but was not sufficient to significantly promote ADSC differentiation into mature endothelial cells in vitro, ie, before injection in the ischemic hindlimb.

Mitochondrial ROS Preconditioning Improves the Paracrine Secretions of ADSCs

Potential paracrine effects of infiltrating ADSCs are likely to contribute to the improved neovascularization after stem cell therapy.[5] We then sought to investigate the effects of mito-

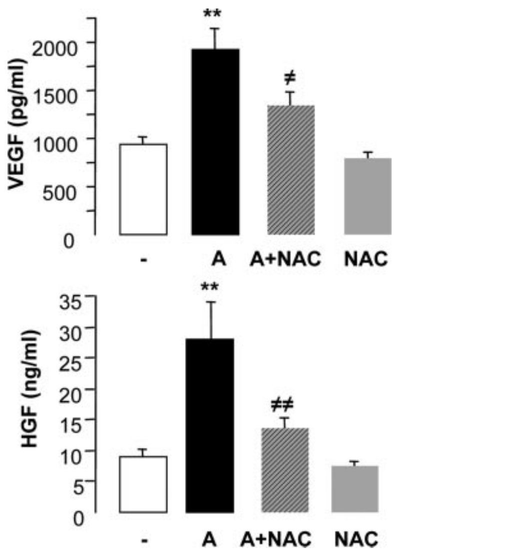

Figure 5. Mitochondrial ROS increase secretion of VEGF and HGF by ADSCs. ADSCs were incubated for 48 hours with antimycin (A) with or without NAC. Quantitative evaluation of VEGF and HGF protein levels in the supernatant of ADSCs were performed. n=5. **$P<0.01$ vs control ADSCs, ≠$P<0.05$ and ≠≠$P<0.01$ vs antimycin-treated ADSCs.

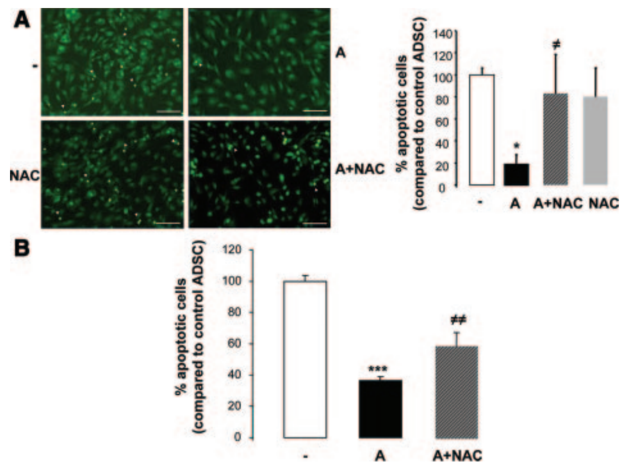

Figure 6. Mitochondrial ROS induce ADSC resistance to H_2O_2-induced cell death. ADSCs were incubated with antimycin (A) with or without NAC for 48 hours. ADSCs were then incubated for 8 hours with medium deprived of serum and supplemented with H_2O_2. A, Immunostaining experiments to detect iodure propidium and SYTO-13 incorporation were performed. White arrows show apoptotic cells. B, Measurement of apoptosis was also performed by detection of Annexin V/iodure propidium by FACS analyses. n=3 (each point in quadruplets). ***$P<0.001$ and *$P<0.05$ vs control ADSCs, ≠≠$P<0.01$ and ≠$P<0.05$ vs antimycin-treated ADSCs. Each bar represents 100 μm.

chondrial ROS preconditioning on the ability of ADSCs to express and produce proangiogenic and prosurvival growth factors. We focused on VEGF and HGF because they are involved in ADSC-mediated vessel growth.[5] VEGF and HGF protein levels were significantly upregulated by 1.9- and 3.4-fold, respectively, in supernatants of antimycin-treated ADSCs compared to control cells ($P<0.01$, Figure 5). Cotreatment with NAC significantly abrogated antimycin-related effects on both VEGF and HGF protein contents. These results demonstrated that mitochondrial ROS preconditioning increased secretion of proangiogenic and prosurvival growth factors by ADSCs.

Mitochondrial ROS Preconditioning Prevents ADSC Apoptosis

Alternatively, mitochondrial ROS preconditioning may affect ADSC cell survival, especially in ischemic area associated with a marked ROS generation. As previously reported, we showed that hindlimb ischemia induced ROS overproduction as revealed by both nitrotyrosine antibodies and DHE staining (data not shown). We hypothesized that this oxidative stress could be at the origin of toxic cell injury leading to death of transplanted cells. To test whether in vitro mitochondrial ROS preconditioning could protect cells from the oxidative stress–induced cell death, we mimicked an oxidative environment by applying medium deprived of serum and containing 75 μmol/L H_2O_2.

Concomitant staining by SYTO and propidium iodide[27] showed the presence of apoptotic cells in control ADSCs (apoptotic nuclei, designed by white arrow, exhibited condensed green-colored chromatin or fragmentation, compared to normal nuclei exhibiting a loose chromatin) after 8 hours of treatment with H_2O_2 (Figure 6A). Few necrotic or postapoptotic necrotic cells were observed. ADSCs pretreated for 48 hours with antimycin showed a very significant reduction in H_2O_2-mediated apoptosis (19% of apoptotic cells in antimycin-treated ADSCs compared to control cells, Figure 6A). A second approach was used to validate these data. Annexin V/propidium iodure quantification by FACS analyses confirmed the strong protective effect of antimycin against H_2O_2-mediated apoptosis (38% of apoptosis in antimycin-treated ADSCs compared to control cells; $P<0.001$, Figure 6B). Both techniques showed that additional presence of NAC partially prevented this antimycin-induced protection against apoptosis (Figure 6A and 6B). The effect of ADSC supernatants was also investigated by assessing H_2O_2-induced cell death of C2181 murine endothelial cells. The conditioned medium of antimycin-treated ADSCs was also efficient to prevent H_2O_2-induced death of endothelial cells. This effect was also reversed by antioxidant treatment (supplemental Figure II).

Finally, we assessed the effect of mitochondrial ROS preconditioning on ADSC survival in vivo, ie, in ischemic area. Three days after induction of ischemia, GFP-ADSCs could be localized in ischemic areas of hindlimb muscles. Cells double-positive for both GFP and CD31 were detected after injection of control ADSCs, suggesting endothelial differentiation. Interestingly, the number of GFP+/CD31+ cells was markedly increased by 1.5-fold in mice transplanted with antimycin pretreated ADSCs (supplemental Figure III). Cotreatment with NAC hampered this antimycin-related effect ($P<0.05$ versus antimycin pretreated ADSCs). Such an increase in the number of GFP+/CD31+ cells may rely on changes in cell proliferation or cell death. We then measured the ratio between ki67+/tunnel+ cells, 7 days after the onset of treatment. Such ratio was highly increased after the injection of antimycin-treated ADSCs. This latter effect was blunted by antioxidant cotreatment (supplemental Figure IV).

Taken together, these results support the hypothesis that in vitro mitochondrial ROS pretreatment confer to ADSCs but also to other cells strong protection against cell death triggered by an oxidative environment.

Discussion

In this study, we demonstrate that a transient exposure of ADSCs to moderate mitochondrial ROS generation before their in vivo transplantation strongly enhances the proangiogenic properties of ADSCs.

Previously, we demonstrated that ADSCs could behave as both adipocyte and vascular progenitors.[4] These cells also secrete angiogenic and antiapoptotic factors, which contribute to their proangiogenic potential.[5,6] This opened numerous physiological and therapeutic perspectives associated with a challenge to understand and manipulate the mechanisms that govern this plasticity. We hypothesized the involvement of mitochondrial ROS in this setting. Indeed, mitochondrial ROS act as antiadipogenic agents on ADSCs suggesting that ROS may affect ADSC function and fate.[23,24]

We induced moderate changes in mitochondrial ROS generation by different pharmacological strategies able to trigger mitochondrial ROS generation in a dose–response manner. O_2^{-} generation is intimately linked to electron transfer through the respiratory chain.[28] We used 2 different mitochondrial inhibitors at equiactive doses, rotenone and antimycin, which inhibit complex I and III, respectively, and induce by 2 different ways accumulation of electrons inside the respiratory chain and subsequently increase generation of $O_2^{\bullet -}$. As the treatment with mitochondrial inhibitors may also have some effect on energetic status as well as nondefined side effects, we develop strategies to delineate the specific effects attributable to mitochondrial ROS. For this purpose, we used 2 different antioxidants, NAC and PDTC, in combination with antimycin and rotenone. NAC and PDTC act by different ways to scavenge ROS: the first one is a glutathione precursor and the second one is a thiol-reductive and iron-chelating agent. The use of these complementary strategies excludes any nonspecific effects associated with the pharmacological treatments and demonstrates the great ability of in vitro mitochondrial ROS generation to stimulate proangiogenic potential of ADSCs in vivo.

We showed that mitochondrial ROS increase HGF and VEGF production, the stimulation of VEGF expression by ROS being already described in others cells.[19] However, according to the complexity of revascularization and the great number of factors secreted by ADSCs, it is likely that paracrine activities may also include the release of other proangiogenic factors such as MMP and IGF1 proteins.[29–32] Interestingly, moderate mitochondrial ROS production strongly protects ADSCs against H_2O_2-mediated cell death. Both effects are consistent with the fact that mitochondrial ROS preconditioned ADSCs enhances the number of endothelial cell–derived ADSCs in ischemic tissue after their engraftment, as reflected by the higher number of GFP/CD31-positive cells in ischemic areas. In this line, the increase in the balance between proliferation and cell death is also increased.

During ischemia and reperfusion in the vascular tissue, massive amounts of ROS are produced and cause significant injury. However, it is now accepted that ROS can trigger in vivo preconditioning protection. In several models of ischemic preconditioning, it has been shown that multiple short exposures to ischemia/hypoxia induce ROS production, which in turn trigger angiogenesis or cell survival through activation of antioxidant defenses and confers both short and long-term protection of tissues against a lethal ischemic insult.[33–38] In the same way, recent reports demonstrated that hypoxia-preconditioned mesenchymal cells from bone marrow enhances survival of implanted cells and therapeutic benefits.[39–42] Our results clearly show that pretreatment of ADSCs with a moderate mitochondrial ROS generation induced a high resistance of ADSCs to cell death induced by a strong oxidative injury. The first exposure to moderate mitochondrial ROS generation might lead to adaptive protective mechanism of ADSCs against a second and higher in vivo oxidative stress, which takes place in the ischemic areas.

This study also suggests that mitochondrial ROS could be considered as a stress signal, which both strongly inhibits adipocyte differentiation of ADSCs and favors angiogenesis. This is consistent with the antiangiogenic and proadipogenic activities displayed by antioxidants.[36,43] Furthermore, mitochondrial ROS generation is proposed as a metabolic sensor to adapt the cell fate to the metabolic environment but also as component of the O_2 sensing mechanism.[19,20,44] Therefore, we can assume that mitochondrial ROS could permit adaptation of ADSC fate and control the enlargement of adipose tissue according to the environmental conditions. In stress conditions, they could inhibit adipocyte differentiation and enhance proangiogenic properties of bipotent progenitors through activation of angiogenic and antiapoptotic growth factors release, promoting endothelial differentiation and protection against injury mediated cell death. The precise molecular mechanisms underlying these mitochondrial ROS-related effects remain to be elucidated. However, a role for the stress responsive and adipogenic repressor CHOP-10/GADD153, strongly correlated with mitochondrial ROS generation (Figure 4B and ref 24), might be suspected.

In conclusion, our results demonstrate that a simple and modest change in mitochondrial function can greatly affect the angiogenic potential of ADSCs and protects them against oxidative stress–induced cell death. This reinforces the importance of mitochondrial ROS in fat development and may pave the way of strategy designed to enhance ADSC therapeutic potential. In addition, we speculate that similar benefit could be obtained with other stem cells used in cell therapy and particularly in the treatment of cardiovascular and ischemia diseases. According to the big challenge caused by the limited availability of some sources of stem cells, this possibility needs to be carefully checked and opens promising perspectives for stem cell therapy.

Acknowledgments

We thank Y. Barreira (Zootechnie Facility, IFR31) for great help with animals care.

Sources of Funding

This work was supported by National Research Program on cardiovascular diseases (No. PNC0402), Cell and Gene therapy program from Région Midi-Pyrénées (No. 03011999), Interreg IIIA (#13 13A-A-7-85-O). A.C. was a fellow of the French Ministère de

l'Education Nationale, de la Recherche, et de la Technologie. S.D. was financially supported by Etablissement Français du Sang, Pyrénées-Méditerrannée. J.S.S. is supported by grants from ANR "young investigator" (JC05-45445) and ANR-05-028-01, Cardiovascular, Obesity, and Diabetes.

Disclosures

None.

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