Abstract: Angiotensin II (Ang II) is a peptide hormone able to elicit a strong production of reactive oxygen species by human neutrophils. In this work, we have addressed whether expression of heme oxygenase-1 (HO-1), an antioxidant enzyme, becomes altered in these cells upon Ang II treatment or under hypertension conditions. In neutrophils from healthy and hypertensive subjects, induction of HO-1 mRNA and protein expression with a parallel increase in enzyme activity took place upon treatment with 15-deoxy-Δ12,14-PGJ2 (15dPGJ2). However, Ang II prevented HO-1 synthesis by normal neutrophils in vitro, and HO-1 expression was depressed in neutrophils from hypertensive patients in comparison with cells from healthy subjects. In addition, Ang II treatment led to a reduced HO-1 enzyme activity to levels similar to those found in neutrophils from hypertensive patients. NO donors reversed the inhibition of 15dPGJ2-dependent HO-1 expression in neutrophils from hypertensive patients, and conversely, inhibition of inducible NO synthase (NOS2) activity counteracted the stimulatory effect of 15dPGJ2 on HO-1 expression in normal human neutrophils. Moreover, Ang II canceled 15dPGJ2-dependent induction of NOS2 mRNA synthesis. Present findings indicate that down-regulation of HO-1 expression in neutrophils from hypertensive subjects is likely exerted through the inhibition of NOS2 expression. Additionally, they underscore the potential usefulness of NO donors as new, therapeutic agents against hypertension. J. Leukoc. Biol. 84: 397–405; 2008.

Key Words: antioxidant enzymes · polymorphonuclear cells · oxidative stress · nitric oxide

INTRODUCTION

Neutrophils constitute one of the main inflammatory cell types, able to respond to a wide variety of stimuli and once activated, to release reactive oxygen species (ROS) and mediators of proteolytic tissue degradation, thereby contributing to oxidative stress, inflammation, and endothelial damage [1, 2]. Oxidative stress may result from an excessive production of ROS or a reduced antioxidant availability. In this light, it has been described that in hypertensive patients, the plasma levels of antioxidants, such as vitamins A and E, and total antioxidant capacity are decreased [3]. Alterations of normal endothelium function appear to play a central role in the pathogenesis of hypertension [4]. Among the mechanisms causing endothelial dysfunction that have been implicated in essential hypertension are oxidative stress and inflammation [5], promoted by endothelial cells themselves or by other blood-circulating cells. Angiotensin II (Ang II), the main peptide hormone of the renin-angiotensin system, elicits leukocyte recruitment to the vessel wall, which constitutes a hallmark of early stages of atherosclerosis and several hypertensive diseases [6, 7]. Angiotensin type 1 (AT1) receptors for Ang II have been found recently to be present in circulating neutrophils [8, 9]. In addition, we have recently described that Ang II highly stimulates endogenous and extracellular superoxide anion radical (O2·−)/ROS production by human neutrophils with associated activation of MAPK [10]. Heme oxygenases (HO) are enzymes catalyzing the conversion of the heme group of hemoglobin into biliverdin and carbon monoxide (CO) [11]. HO-1 is an inducible isoform, whose expression is up-regulated in response to various stimuli [12]. Recently, we have shown that HO-1 expression is induced by 15-deoxy-Δ12,14-PGJ2 (15dPGJ2) or sodium arsenite (NaAsO2) in human lymphocytes [13]. Induction of HO-1 expression leads to depletion of cytochrome P450 mono-oxygenase, whose products contribute to hypertension [14]. HO-1 also decreases the cellular levels of heme, a pro-oxidant group, and elevates those of bilirubin, an antioxidant [15]. As well, an important, protective function has been assigned to products of HO-1 enzyme activity as inhibi-
tors of hypoxia-induced vasoconstrictory and proinflammatory pathways [16]. Thus, induction of HO-1 expression is currently considered as an important mechanism to counteract intracellular oxidative stress. In this context, it has been described that HO-1 expression becomes activated by pro-oxidant reagents via translocation to the nucleus of the transcription factor NF-E2-related factor-2 (Nrf2) [17].

Conflicting results have been reported about HO-1 expression in the hypertensive state in different rat and human tissues, with levels of HO-1 protein found enhanced [18, 19] or decreased [20, 21]. Also, there is increasing evidence that gas monoxides, such as NO and CO, play a substantial role as modulators of vascular tone [22]. NO is generated from l-arginine by NO synthase (NOS), present in its inducible isoenzyme (NOS2) in neutrophils and in constitutive isoforms in smooth muscle and endothelial cells [22].

Here, we have investigated HO-1 expression in human normal neutrophils under treatment with Ang II, as well as in neutrophils from hypertensive patients. We show evidence that in both instances, neutrophils display a reduced HO-1 expression at mRNA and protein levels, together with a decreased enzyme activity and that in these cells, HO-1 levels are modulated by NO.

MATERIALS AND METHODS

Isolation and culture of human neutrophils

Human peripheral blood neutrophils were isolated and cultured as indicated [23].

Subjects

Patients were eligible as hypertensive if they were >18 years old, suffered from moderate-to-high hypertension (systolic blood pressure, >140 mm Hg; diastolic blood pressure, >90 mm Hg; both measured for 24 h of Holter monitoring during unrestricted daily life), and had not undergone any previous treatment with antihypertensive drugs or other compounds. Patients with diabetes mellitus, fasting glucose in serum >120 mg/dL, total cholesterol levels >240 mg/dL, other pathologies were excluded. The criteria for normotensive subjects were to be under antihypertensive drugs or other compounds. Patients with hypertension were treated with angiotensin receptor blockers (Merck, Rahway, NJ, USA; 50–100 mg/day) for 1 year on average. Upon treatment, they showed a decrease of their blood pressure until reaching normotensive values and did not experience any secondary effects. Each subject gave informed consent prior to its undertaking.

Western blotting analysis of HO-1 and Nrf2 protein levels

Total cell lysates and nuclear extracts were prepared basically as described [23]. Western blotting analysis of HO-1 protein levels was performed on total cell lysates as described [13]. To verify even protein loading, the blots were subsequently stripped and reprobed with polyclonal antibodies against GAPDH at a 1:1000 dilution. Nrf2 levels were analyzed on nuclear extracts by subsequent stripping and reprobed with polyclonal antibodies against Nrf2 IgG from Santa Cruz Biotechnology (Santa Cruz, CA, USA) at a 1:1000 dilution. Band intensities were quantitated densitometrically using the Scion Image software (Scion Inc., Frederick, MD, USA) and corrected, in the case of HO-1, for differences in GAPDH levels.

RT-PCR analysis of NOS2 and HO-1 mRNA levels

Total cellular RNA extraction and RT into cDNA were performed as described previously [13]. NOS2 transcript levels were quantitated after PCR amplification as indicated [24] and corrected for differences in GAPDH expression. Real-time PCR analysis of HO-1 mRNA levels was performed using the ABI Prism 7300 sequence detection system from Applied Biosystems (Foster City, CA, USA), under the specific thermocycler conditions recommended by the manufacturer for the primers used. PCR reactions were performed in triplicate. Each sample was also analyzed for GAPDH transcript levels to normalize for RNA input amounts. For the relative quantification of gene expression, the comparative threshold cycle method was used as described in the ABI Prism 7700 User Bulletin 2.

Measurement of HO-1 activity

HO-1 activity was measured by determining the rate of bilirubin production according to the procedure described previously [25].

Detection of cellular apoptosis

Apoptosis, measured as DNA fragmentation, was tested using the Cell Death Detection ELISA kit from Roche Applied Science (Barcelona, Spain), according to the manufacturer’s instructions. No evidence of apoptotic death was found in our cells, even after 15 h of incubation in our experimental conditions.

Statistical analysis

mRNA and protein expression levels are expressed in arbitrary units as the mean ± SEM from a minimum number of three independent experiments. The data were subjected to ANOVA and the Student’s paired t-test using GraphPad Prism 5.0 software. Symbols (*, **, and †) indicate a P value <0.01.

RESULTS

Ang II inhibits HO-1 expression in a time- and dose-dependent manner in human neutrophils

To investigate the effect of Ang II on HO-1 expression in human neutrophils, cells from healthy subjects were incubated with Ang II prior to treatment with two well-known inducers of HO-1 expression, 15dPGJ2 (Fig. 1A) or NaAsO2 (Fig. 1B). Basal levels of HO-1 protein were undetectable in untreated cells. However, when they were stimulated in vitro with 15dPGJ2 (Fig. 1A) or with NaAsO2 (Fig. 1B) for 5 h, synthesis of HO-1 was clearly detected. Figure 1A shows that Ang II prevented HO-1 protein expression induced by 15dPGJ2 in a dose- and time-dependent manner. This inhibition was complete when neutrophils were treated with Ang II (100 nM) for 90 min prior to 15dPGJ2 addition. Figure 1B shows that Ang II exerted a similar inhibitory effect on cells stimulated with NaAsO2.

It has been described that an elevation of HO-1 expression can take place in the liver without a parallel increase in HO enzyme activity [26]. However, the analysis of HO-1 activity in human neutrophils incubated with 15dPGJ2 revealed that its levels were increased significantly (to 6.72±0.21 nmol/mg protein/h; n=4) compared with untreated cells (1.03±0.15 nmol/mg protein/h; n=4). As well, Ang II pretreatment for 90 min inhibited the positive effect of 15dPGJ2 (to 3.01±0.18 nmol/mg protein/h; n=4) on HO-1 enzyme activity.

Nuclear translocation of the transcription factor Nrf2 is inhibited by Ang II

Next, experiments were conducted to analyze the possible participation of Nrf2 in 15dPGJ2- and NaAsO2-dependent HO-1 expression. As shown in Figure 2A, these two compounds were able to elicit by themselves Nrf2 nuclear translocation. Moreover, Ang II treatment prior to 15dPGJ2 or

NaAsO₂ addition significantly prevented nuclear accumulation of Nrf2.

Ang II-negative effect on HO-1 expression is MAPK- and ROS-independent

We have demonstrated previously that Ang II elicits the activation of protein kinases of the MAPK family in human neutrophils [10], which led us to address their potential implication in HO-1 down-regulation by Ang II. Although the specific MAPK inhibitors, PD098059 and SB203580, and the specific PI-3K inhibitor LY294002 were found to inhibit the stimulation of HO-1 expression elicited by 15dPGJ₂, they did not alter, however, the negative effect of Ang II on HO-1 expression (data not shown), as it was expected.

Ang II treatment has been shown to rapidly (i.e., within minutes) promote ROS production through NADPH oxidase activation in human neutrophils [10]. Subsequent experiments were thus addressed to examine the effect of the pretreatment of cells with DEM or NAC, two compounds with pro-oxidant and antioxidant activities, respectively, on the long-term inhibition exerted by Ang II on HO-1 expression. As shown in Figure 2B, neutrophils preincubated with DEM experienced a significantly higher 15dPGJ₂-induced HO-1 expression than did DEM-untreated cells, whereas NAC preincubation inhibited 15dPGJ₂-dependent stimulation of HO-1 expression. Regarding their actions on the negative effect of Ang-II on HO-1 expression, we found that whereas DEM partially counteracted the action of Ang II, NAC did not prevent such effect of this hormone. These results suggested that the long-term, down-modulatory action of Ang II on HO-1 expression was not dependent on ROS levels.

Antihypertensive agents counteract the inhibitory effect of Ang II on HO-1 expression

To further characterize the Ang II inhibitory effect on HO-1 protein expression, normal human neutrophils were preincubated separately in the presence of two different antihypertensive agents, namely, losartan, a selective AT1 receptor antagonist, and hydralazine, a vasodilator drug. Figure 3A illustrates that these two compounds were, by themselves, able to
partially relieve the negative effect exerted by Ang II on 15dPGJ2-dependent HO-1 expression. As well, losartan elicited a similar preventive effect on HO-1 induction promoted by NaAsO2 (Fig. 3B). Neither losartan (Fig. 3B) nor hydralazine (data not shown) was able, however, to elicit HO-1 expression by itself. The results obtained with losartan thus indicated that Ang II negatively modulates HO-1 expression through specific Ang II receptor-dependent pathways. On the other hand, the hydralazine effect shown in Figure 3A suggested that other cellular mechanisms could be involved as well in the Ang II effect.

Induction of HO-1 expression is a NO-dependent process

The antihypertensive effect of hydralazine could be exerted by promoting an increase of intracellular cGMP levels [27], in similarity to the mode of action of NO. This prompted us to investigate the potential effect of NO on the Ang II inhibitory effect on HO-1 expression. Previously, it has been shown that NO is a relevant, endogenous inducer of HO-1 in endothelial cells [28]. In subsequent experiments, we thus explored the potential interplay between NO and HO-1 expression in neutrophils. We found that the NO donors, diethylenetriamine (DETA)-NO and sodium nitroprusside (SNP), were separately able to stimulate HO-1 expression in human neutrophils (Fig. 4A). However, when these cells were pretreated with Ang II, the positive effect of these compounds on HO-1 expression was partially prevented (Fig. 4A). In an opposite way, when neutrophils were incubated with the inhibitor of NOS2 activity, L-NMMA, prior to the addition of NaAsO2 or 15dPGJ2, the enhancing effect exerted by these two compounds on HO-1 expression was canceled almost completely when 15dPGJ2 was

Fig. 3. Antihypertensive drugs reverse the inhibitory effect of Ang II on HO-1 expression in human neutrophils, which were preincubated at 37°C in the absence or presence of losartan (20 μg/mL; A and B) or hydralazine (100 μM; A) for 30 min and then treated with Ang II at the indicated doses (A) or at 100 nM (B) for 90 min. Thereafter, the cells were incubated for 5 h in the absence or presence of 15dPGJ2 (10 μM; A) or NaAsO2 (5 μM; B), and HO-1 levels were analyzed on cell lysates by Western blotting. Each blot is representative of a set of three separate experiments yielding similar results. Plotted values represent HO-1 protein expression (mean ± SEM) corrected for differences in GAPDH levels. *, P < 0.01, for 15dPGJ2-stimulated, Ang II-treated versus Ang II-untreated; **, P < 0.01, for 15dPGJ2- or NaAsO2-stimulated plus Ang II-, losartan-, or hydralazine-treated versus losartan- or hydralazine-untreated.

Fig. 4. Induction of HO-1 expression and Nrf2 nuclear translocation in normal neutrophils is dependent on NOS2 activity. Neutrophils were preincubated at 37°C in the absence or presence of Ang II (100 nM) for 90 min and then treated with DETA-NO (1 mM) or SNP (0.25 mM) for 5 h (A). Neutrophils were preincubated, with or without N′-methyl-L-arginine acetate (L-NMMA; 0.6 mM), for 30 min (B and C) and then treated or not with Ang II (100 nM) for 90 min (C) and thereafter, stimulated with NaAsO2 (5 μM) or 15dPGJ2 (10 μM) for 5 h (B and C). HO-1 levels were analyzed on cell lysates by Western blotting (A–C). Neutrophils were preincubated, with or without Ang II (100 nM), for 30 min and then treated with DETA-NO (1 mM) for 30 min. Nrf2 levels were analyzed on nuclear extracts by Western blotting (D). Each blot is representative of a set of three separate experiments yielding similar results. Plotted values (mean ± SEM) represent HO-1 protein expression corrected for differences in GAPDH levels (A–C) or Nrf2 nuclear protein levels (D). *, P < 0.01, for NO donor-treated, Ang II-treated versus Ang II-untreated (A and D), or P < 0.01 for 15dPGJ2- or NaAsO2-stimulated, Ang II- and/or L-NMMA-treated versus Ang II- and/or L-NMMA-untreated (B and C).
the stimulus and was reduced significantly in NaAsO\textsubscript{2}-treated neutrophils (Fig. 4B). The inhibition of NaAsO\textsubscript{2}-promoted HO-1 expression was potentiated by the simultaneous presence of L-NMMA and Ang II (Fig. 4C). Additionally, we found that the treatment of cells with DETA-NO promoted Nrf2 translocation and that this process was inhibited as well by prior treatment of neutrophils with Ang II (Fig. 4D). These results suggested that NO could be a pivotal signaling molecule mediating 15dPGJ\textsubscript{2}- and NaAsO\textsubscript{2}-dependent induction of HO-1 expression.

**Effect of Ang II on NOS2 mRNA levels in human neutrophils**

Experiments were also undertaken to address whether 15dPGJ\textsubscript{2} and NaAsO\textsubscript{2} were able to activate NOS2 expression in human neutrophils. Previous studies, in keeping with our own experience, have shown the difficulty to detect released NO and the presence of the NOS2 protein in human neutrophils [29]. However, we were able to readily detect the NOS2 transcript by means of RT-PCR using the experimental conditions described by Wheeler et al. [24]. Figure 5A illustrates that 15dPGJ\textsubscript{2} or NaAsO\textsubscript{2} treatment induced a strong transcription of the NOS2 gene. In concordance with the negative effects of Ang II on HO-1 shown above, 15dPGJ\textsubscript{2}-promoted NOS2 mRNA transcription was canceled as well in neutrophils pre-treated with this peptide hormone (Fig. 5B).

**HO-1 expression is depressed in human neutrophils from hypertensive patients**

We compared the capacity to express HO-1 by neutrophils isolated from healthy subjects and hypertensive patients. As previously observed, basal levels of HO-1 protein were undetectable in cells from both sources (Fig. 6, A and C). However, when they were stimulated in vitro with 15dPGJ\textsubscript{2} (Fig. 6A) or with NaAsO\textsubscript{2} (Fig. 6C), synthesis of this protein was clearly detected. However, it was noteworthy that HO-1 expression in the presence of these inducers was markedly lower in neutrophils obtained from hypertensive patients than in cells from healthy subjects (Fig. 6, A and C). This reduced induction of HO-1 expression at the protein level strongly correlated with lower HO-1 mRNA levels being found in neutrophils isolated from hypertensive patients compared with levels in cells from...
Inhibition of HO-1 expression is relieved in neutrophils from drug-treated, hypertensive patients

To study the effect of treatment with antihypertensive compounds on HO-1 expression, the above hypertensive patients were clinically treated with the Ang II receptor blocker, losartan (50–100 mg/day), for 1 year on average. As shown in Figure 7A, HO-1 protein levels in neutrophils from losartan-treated, hypertensive patients, incubated in the absence or presence of 15dPGJ2 for 15 h, were similar to those found in neutrophils from healthy subjects. In Figure 7B neutrophils from both origins were incubated or not with 15dPGJ2 for 4 h to quantitate HO-1 mRNA levels, and results obtained correlated with HO-1 protein levels. Figure 7C shows statistical data from experiments addressing HO-1 protein expression levels in human neutrophils from healthy and treated hypertensive patients, and Figure 7D presents HO-1 mRNA levels measured by real-time RT-PCR in neutrophils from both groups. Moreover, HO-1 in neutrophils from hypertensive patients treated with 15dPGJ2 showed a decreased enzyme activity (1.81±0.34 nmol/mg protein/h; n=4) compared with levels in neutrophils from healthy subjects (6.72±0.21 nmol/mg protein/h; n=4). This down-regulatory effect of hypertension on HO-1 expression seemed specific for neutrophils as circulating cells, as in peripheral blood lymphocytes from hypertensive patients, it was not observed (data not shown). Finally, it should be mentioned that in addition to HO-1, the enzymatic activity of glutathione peroxidase (GPx), which plays a central role in protecting cells from oxidative injury, was depressed in neutrophils from hypertensive patients to 21.3% (n=9) of activity levels found in cells from healthy subjects (G. Alba and F. Sobrino, manuscript in preparation). Recent reports have also shown that GPx-1 activity is reduced in mononuclear cells from hypertensive subjects [30].

Normotensive subjects. As shown (Fig. 6, B and D), whereas in neutrophils from healthy subjects, the HO-1 mRNA transcript was clearly detectable after 4 h of 15dPGJ2 or NaAsO2 treatment, neutrophils from hypertensive patients did not respond significantly to any of these two stimuli. For the sake of graphic illustration, Figure 6E shows statistical data from experiments addressing HO-1 protein levels in human neutrophils from healthy and hypertensive patients, and Figure 6F shows HO-1 mRNA levels measured by real-time RT-PCR from neutrophils from both groups. Moreover, HO-1 in neutrophils from hypertensive patients treated with 15dPGJ2 showed a decreased enzyme activity (1.81±0.34 nmol/mg protein/h; n=4) compared with levels in neutrophils from healthy subjects (6.72±0.21 nmol/mg protein/h; n=4). This down-regulatory effect of hypertension on HO-1 expression seemed specific for neutrophils as circulating cells, as in peripheral blood lymphocytes from hypertensive patients, it was not observed (data not shown). Finally, it should be mentioned that in addition to HO-1, the enzymatic activity of glutathione peroxidase (GPx), which plays a central role in protecting cells from oxidative injury, was depressed in neutrophils from hypertensive patients to 21.3% (n=9) of activity levels found in cells from healthy subjects (G. Alba and F. Sobrino, manuscript in preparation). Recent reports have also shown that GPx-1 activity is reduced in mononuclear cells from hypertensive subjects [30].

Inhibition of HO-1 expression is relieved in neutrophils from drug-treated, hypertensive patients

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DISCUSSION

The present study was designed on the basis of two facts. The first is the important role of oxidative stress and inflammation in the pathogenesis of vascular disease and hypertension [31], which may be mediated or promoted by circulating blood cells [32]. The second is that up-regulation of HO-1 expression is currently well-accepted as a protective mechanism against intracellular oxidative stress [33]. To explain the enhanced oxidant activity in neutrophils in the hypertensive state, the observation is interesting that levels of arachidonic acid, a well-known activator of NADPH oxidase and precursor of proinflammatory PGs, are increased during essential hypertension in neutrophils [34].

Expression of HO-1 in neutrophils has been reported recently, together with the inhibitory role that HO-1 overexpression exerts on the cascade of host inflammatory responses mediated by neutrophils and macrophages [35].

By using 15dPGJ2 or NaAsO2, two strong stimulators of HO-1 expression in lymphocytes [13], we were able to detect that HO-1 mRNA and protein synthesis, together with HO-1 activity, were down-regulated in neutrophils from healthy subjects treated with Ang II. It is noteworthy that effects similar to those found in Ang II-treated neutrophils were observed in cells from hypertensive patients. Moreover, we found that this inhibition of HO-1 expression was reversed in neutrophils from hypertensive patients treated with antihypertensive drugs. In the same line, other groups have reported a decreased HO-1 expression in the pulmonary artery of young rats in hypertension [20] and in Ang II-treated rat vascular smooth muscle cells [36]. Yet, opposite results in the aorta [18] and liver [19] of Ang II-treated rodents have also been reported. Present results are in agreement with recent data showing that a number of enzymes relevant for the defense against oxidative stress exhibit a decreased activity and mRNA level in mononuclear cells of hypertensive subjects and that their levels become increased in vivo after treatment of these patients with antihypertensive drugs [30].

In light of mechanisms accounting for the inhibitory effect of Ang II on HO-1 expression, several possibilities can be considered. We have previously demonstrated that Ang II strongly triggers ROS production by human neutrophils [10]. This finding raises the possibility that the Ang II-negative effect on HO-1 expression could be mediated by ROS production. However, this hypothesis was unlikely, as neither NAC nor DEM, two compounds exerting on cell antioxidant and pro-oxidant actions, respectively, was able to modify the down-regulation by Ang II of 15dPGJ2-elicted HO-1 synthesis. Besides, the inhibitory action of Ang II on HO-1 transcription was detected in the long term, i.e., within several hours, whereas the stimulation of Ang II on NADPH oxidase, reported previously by us [10], took place in the short term, i.e., within minutes. Another possibility was the potential implication of NO in the Ang II-negative effect on HO-1 expression. The relationship among HO-1 expression, NO levels, and hypertension is complex and seems to be tissue-specific. In this context, NO antagonizes the effects of Ang II, whereas the latter decreases NO bioavailability [37]. We observed that transcription of the NOS2 gene is induced by 15dPGJ2 in neutrophils. Moreover, we found evidence of a modulatory link between HO-1 and NOS2, as HO-1 protein expression, induced by 15dPGJ2 or NaAsO2, became hindered when NOS2 activity was inhibited. These data are in agreement with previous observations about the essential role of NOS2 activity on HO-1 expression in endothelial cells [38, 39]. In addition, we found that NO donors were able to induce HO-1 protein expression and Nrf2 translocation to the nucleus in human neutrophils and that both processes were severely hampered in Ang II-treated neutrophils from normotensive subjects, as was the case for untreated neutrophils from hypertensive patients. Thus, our data are in concordance with previous results showing that NOS2 activity is decreased in neutrophils from hypertensive subjects [40]. In an opposite way, in the aorta from hypertensive rats, up-regulation of HO-1 and NOS2 expression has been found [41]. Other authors have reported that overexpression of HO-1 suppresses the vaso-dilatory response to NO by vascular smooth muscle cells [42]. Present data suggest the positive effect of 15dPGJ2 on NOS2 expression in human neutrophils to be rather specific for this cell type, as in astrocytes [43] and kidney cells [44], 15dPGJ2 has been reported instead to decrease NOS2 expression levels significantly.

In the context of the possible physiological relevance of the present findings, we believe that oxidative stress must be considered as a crucial mechanism operative in pathologies such as atherosclerosis and hypertension. In addition to the proinflammatory effects exerted by Ang II on neutrophils in the short term, this hormone might contribute to injury of the vessel wall by decreasing in the long term the expression levels of HO-1, which acts as a protective antioxidant enzyme. In this context, other authors have described that HO-1 products, i.e., biliverdin and CO, inhibit neutrophil migration [45]. Therefore, the inhibition of HO-1 expression found in hypertension, by resulting in a reduced release of such products, could contribute to potentiate the inflammatory process. Moreover, we found such depressed HO-1 expression to become relieved after clinical, antihypertensive treatment of patients. In this line, accelerated atherosclerotic lesion is enhanced in HO-1 null mice compared with wild-type animals. In contrast, transgenic mice with cardiac-specific overexpression of HO-1 exhibit an improvement in cardiac function [46, 47]. Also, in this light, in the renal system, chemical induction of HO-1 expression has been shown to attenuate the development of renovascular hypertension, whereas the treatment with a HO-1 inhibitor led to increased blood pressure [48].

In summary, the impact of ROS products released by neutrophils may well contribute, in our view, to the endothelial dysfunction and organ damage experienced by hypertensive subjects. This work thus offers new insight into the essential role played by NO in counteracting the reduced HO-1 expression associated with the hypertensive state and/or elicited by Ang II in human neutrophils. Research about the potential usefulness of NO donors as new, effective therapeutic agents against pathological hypertension should therefore be warranted.
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