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News & Views

Discordant Response of Glutathione and Thioredoxin Systems in Human Hypertension?

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ABSTRACT

Hypertension is frequently associated with oxidative stress caused by high production of reactive oxygen species and compromised antioxidant defenses. Humans with essential hypertension, with or without treatment, and controls were examined (35 hypertensive and 30 normotensive). We noted a discordant response of the glutathione and thioredoxin systems in essential hypertension and to antihypertensive treatment. Further studies examining the significance of these thiols in hypertension outcomes are warranted. *Antioxid. Redox Signal.* 9, 507–514.

OXIDATIVE STRESS IN HYPERTENSION

The importance of oxidative stress (OS) in hypertension has received increasing attention in the last few years. It seems to contribute to maintaining elevated blood pressure values (25, 27, 30) and to developing hypertension-induced organ damage (12, 17). Marked increases in OS levels, as well as in reactive oxygen species (ROS) by-products and reduced activity of some of the antioxidant mechanisms have been described in essential hypertension (18). Moreover, antihypertensive treatment was able to reduce OS close to normal levels (20). Although the increased ROS generation has been firmly established from the beginning, several studies have demonstrated that OS in hypertension was the consequence not only of an increase in ROS production but also of an inadequate response of some of the antioxidant mechanisms (2). A growing interest exists in better understanding the behavior of antioxidant mechanisms in hypertension.

GLUTATHIONE AND THIOREDOXIN SYSTEMS

Cellular and blood redox balance is maintained by the coordinate activity of several antioxidant mechanisms. Glutathione (GSH) and thioredoxin (Trx), two thiol-reducing systems, play a key role in defense against excessive ROS generation. These systems participate not only in antioxidant defense, but also in redox regulation of signal transduction, transcription, cell growth, and apoptosis (7, 21). Glutathione, a peptide synthesized by glutamate cysteine ligase (GCL), composed of two subunits (GCLC and GCLM), and of glutathione synthetase (GSS). GSH is used by glutathione peroxidases (GPXs) to reduce peroxides and producing glutathione disulfide or oxidized (GSSG). GPXs are selenium-dependent proteins codified by six different genes (*GPX1* to *GPX6*) in humans, which occupy different locations in cells and tissues. Furthermore, glutathione reductase (GSR) reduces GSSG to GSH.

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Trx is a small dithiol–disulphide protein with two isoforms codified by two different genes, *TRX1* and *TRX2*. Trx1 is present in cytoplasm, nucleus, and extracellular space, whereas Trx2 is present in mitochondria. Oxidized Trx is reduced by thioredoxin reductases (TrxRs), which are codified by the *TXRND1*, *TXRND2*, and *TXRND3* genes. TrxRs are selenoproteins with specific localization in cells and tissues. TrxR1 has a similar distribution to Trx1, TrxR2 similar to Trx2, and TrxR3 has been recently characterized and is present in a small number of tissues (10). Regulation of these systems is complex, and they are reciprocally regulated (5, 8, 15). In general, the Trx system is enhanced when the major intracellular antioxidant GSH is consumed by oxidative stress, although impaired induction of Trx in tissues from genetically hypertensive rats, despite the relative increment of oxidative stress, has been described (22). Genetic expression of these systems seems to be independent, but many times, their mRNA levels are modified in the same way by different agents (5, 8).

Although abnormalities in GSH levels and in other components of the GSH system have been analyzed in hypertension (2), little information exists about the status of the Trx system (11), and overall, about their gene-expression modification.

GENERAL CHARACTERISTICS OF THE STUDY POPULATION

The study was performed on 35 hypertensive patients in the absence of antihypertensive treatment and after 3 months of treatment and on 30 control volunteers. The characteristics of patients and controls are shown in Table 1. Controls were normotensive and had slightly lower total cholesterol levels than did the hypertensive patients. Antihypertensive treatment during 3 months significantly reduced both systolic and diastolic BP levels for both office BP (from 155 ± 3.1/97.6 ± 2.2 to 135.4 ± 3.2/86.3 ± 3.1 mm Hg; $p < 0.001$), as well as for the average of 24-h ambulatory BP (from 140.2 ± 2.1/89.9 ± 1.6 to 125.3 ± 2.9/77.9 ± 1.4 mm Hg; $p < 0.001$). The changes were similar in subjects receiving nonpharmacologic treatment, an angiotensin-receptor blocker–based treatment, and in those with a β-blocker–based therapy (data not shown).

OXIDATIVE STRESS, GLUTATHIONE, AND THIOREDOXIN IN HYPERTENSIVE PATIENTS AND CONTROLS

Untreated hypertensive patients had lower levels of GSH, higher levels of GSSG, and a higher GSSG/GSH ratio in plasma compared with normotensive controls (Table 3). No relation, however, exists between GSH or GSSG/GSH ratios and SBP or DBP values, either in the office or as an average of 24-h ambulatory values. No differences were found for Trx values between untreated hypertensive patients and normotensive controls (see Table 2). During the antihypertensive treatment, GSH levels increased significantly, whereas GSSG levels and the GSSG/GSH ratio were reduced, and no changes were observed in Trx. Among the treatment groups, no differences in GSH and GSSG levels and the GSSG/GSH ratio were present (data not shown).

Mononuclear cells from hypertensive subjects showed significantly lower GSH but higher GSSG and GSSG/GSH values than did the control group (see Table 2). Likewise, 8-oxo-dG and MDA, by-products of ROS-induced DNA damage and lipid peroxidation, respectively, were also significantly increased in hypertensive subjects as compared with the control group. Antihypertensive treatment for 3 months reduced the OS, measured as the GSSG/GSH ratio, and the ROS-induced by-products (see Table 2).

Table 1. General Characteristics of the Study Population

	Controls (n = 30)	p[a]	*Hypertensives (n = 35)*	p[b]	*Hypertensives treated (n = 35)*
Age (yr)	43.2 ± 9.3	ns	46.1 ± 1.5		
Sex (M/F)	17/13	ns	19/20		
Body mass index (kg/m^2)	26.3 ± 3.8	ns	29.3 ± 0.5	ns	28.9 ± 0.9
Office SBP (mm Hg)	127.6 ± 10.3	$p < 0.001$	155.1 ± 3.1	$p < 0.001$	135.4 ± 3.2
Office DBP (mm Hg)	75.9 ± 7.0	$p < 0.001$	97.6 ± 2.2	$p < 0.001$	86.3 ± 3.1
24-h SBP (mmHg)	—	—	140.2 ± 2.1	$p < 0.001$	125.3 ± 2.9
24-h DBP (mm Hg)	—	—	89.9 ± 1.6	$p < 0.001$	77.9 ± 1.4
Baseline glucose (mg/dl)	96.1 ± 1.2	ns	103.1 ± 3.2	ns	99.4 ± 1.6
Total cholesterol (mg/dl)	196.3 ± 3.2	ns	206.1 ± 4.2	ns	208.2 ± 4.0
HDL-cholesterol (mg/dl)	48.2 ± 0.5	ns	44.0 ± 1.2	ns	44.3 ± 1.3
Triglycerides (mg/dl	121.8 ± 9.2	ns	137.2 ± 9.9	ns	144.3 ± 10.1
Menopause	0		3		3

Values are expressed as mean ± standard error of the mean.

[a]Values denote differences between hypertensive patients and controls.

[b]Values denote differences between hypertensive patients in the absence of and after 3 months of antihypertensive treatment.

TABLE 2. OXIDATIVE STRESS PARAMETERS IN BLOOD AND MONONUCLEAR CELLS

	Controls (n = 30)	p[a]	*Hypertensives (n = 35)*	p[b]	*Hypertensives treated (n = 35)*
Plasma					
GSH, µmol/g Hb	5.6 ± 0.3	<0.001	3.3 ± 0.1	<0.001	6.5 ± 0.1
GSSG, µmol/g Hb	1.4 ± 0.2	<0.001	3.91 ± 0.08	<0.001	2.3 ± 0.09
GSSG/GSH	0.22 ± 0.1	<0.001	1.33 ± 0.1	<0.001	0.37 ± 0.05
MDA, µmol/g Hb	0.18 ± 0.02	<0.001	0.84 ± 0.04	<0.001	0.29 ± 0.08
Trx, ng/ml	19.6 ± 0.6	ns	18.5 ± 0.5	ns	20.2 ± 0.6
Mononuclear cells					
GSH, µmol/mg prot	25.1 ± 1.7	<0.001	15.8 ± 0.5	<0.001	24.1 ± 0.4
GSSG, µmol/mg prot	0.1 ± 0.02	<0.001	1.1 ± 0.05	<0.001	0.4 ± 0.01
GSSG/GSH	0.01 ± 0.01	<0.001	0.08 ± 0.01	<0.001	0.02 ± 0.02
MDA, µmol/mg prot	0.14 ± 0.1	<0.001	0.73 ± 0.2	<0.001	0.32 ± 0.09
8-oxo-dG/dG nDNA	3.0 ± 0.3	<0.001	6.94 ± 0.15	<0.001	4.49 ± 0.12

Values are expressed as mean ± standard error of the mean.

GSH, gluthation reduced; GSSG, gluthation oxidized; MDA, malondialdehide; TRX, thioredoxin; Hb, hemoglobin; 8-oxo-deoxiguanosine is expressed as the number of oxidized bases/10^6 deoxiguanosine; prot, protein.

[a]Differences between normotensive and hypertensive patients in the absence of antihypertensive treatment.

[b]Differences between initial and 3-month evaluation of antihypertensive treatment.

mRNA LEVELS OF ANTIOXIDANT GLUTATHIONE AND THIOREDOXIN SYSTEM COMPONENTS

The mRNA levels of enzymes that control synthesis, regeneration, and utilization of GSH in mononuclear cells are shown in Fig. 1. In hypertensive subjects, mRNA levels of *GSS*, *GSR*, *GCLC*, *GCLM*, *GPX1*, *GPX4*, and *GPX6* genes were significantly lower than those observed in cells from controls. Only mRNA levels of *GPX2* were not different between hypertensives and controls. No relation exists between BP values and the mRNA levels analyzed. During antihypertensive treatment, when OS was reduced, the mRNA levels of the genes of these GSH system enzymes also decreased (see Fig. 1). No changes were observed in the mRNA of *GCLC*, *GCLM*, and *GPX2*. In all genes, the mRNA levels after treatment were lower than those in controls, except for the *GPX2* gene.

In contrast, variations in the levels of the mRNA from genes of the Trx system behaved differently (Fig. 2). At baseline, *TRX1* and *TRX2* mRNA levels as well as *TRXR1*, *TRXR2*, and *TRXR3* levels were significantly higher in hypertensive patients compared with those for controls. These mRNA levels decreased during antihypertensive treatment, although higher mRNA levels remained as compared with those for controls. Only the mRNA of *TRXR3* did not change during the treatment (see Fig. 2).

DIFFERENCES BETWEEN GSH AND TRX SYSTEMS IN HYPERTENSION AND UNDER TREATMENT

The present study was designed simultaneously to assess the status of the two main antioxidant systems, GSH and Trx, in hypertensive subjects in the absence of and during antihypertensive treatment. In hypertensive patients at baseline, in the presence of chronic OS, a great disparity between the two systems was observed in plasma. Whereas GSH levels were significantly lower than they were in controls, no differences were observed for the Trx levels. Reducing blood pressure values with antihypertensive treatment was accompanied by a significant increase of GSH levels, reaching values near to those of the control subjects. Trx values, however, were not modified during treatment.

Discrepancies in the mRNA levels of the components of GSH and Trx systems were also observed. Besides the low levels of GSH, lower mRNAs levels of GSR, GSS, GCLC, and GCLM were observed in mononuclear cells of hypertensive subjects as compared with controls. If the mRNA of the enzymes that tended to maintain GSH levels were reduced, it might have contributed to the low GSH values observed. These low mRNA levels decreased even more under antihypertensive treatment when the OS decreased and the GSH increased, possibly because the GSH requirements were lower than they were in the absence of treatment. The data may indicate that in untreated hypertensives, the expression of the GSH system was the highest possible and that reducing OS by antihypertensive treatment reduces the induction of these genes. These data confirmed previous data in that OS in essential hypertension is dependent not only on an increased ROS production but also on the abnormal response of the GSH antioxidant mechanisms (2).

In contrast to the GSH system, mRNA levels of *TRX1* and *TRX2*, as well as of two of the thioredoxin reductases, *TRXR1* and *TRXR2*, were significantly higher in hypertensive patients than they were in controls. When treatment reduced OS, the mRNA levels of these genes decreased in parallel with the OS, but they are maintained at higher levels than in controls. These data may reflect that with chronic OS, the Trx system was responding by increasing the gene expression. Lowering OS reduced the level of stimulation, a similar behavior pattern to that observed in rapidly induced OS (4, 5).

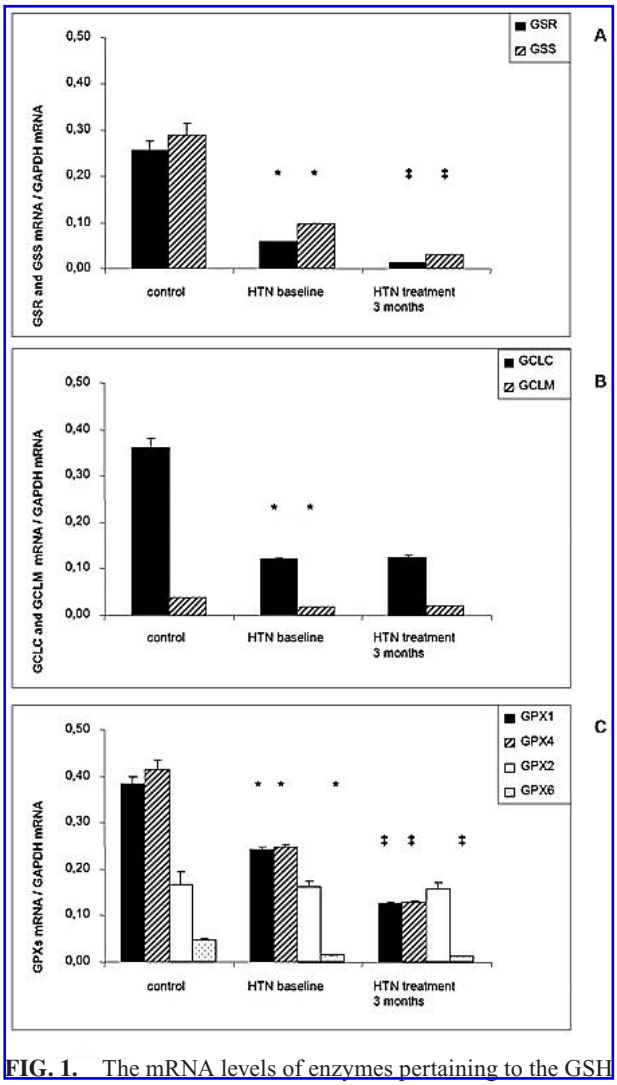

FIG. 1. The mRNA levels of enzymes pertaining to the GSH system in mononuclear cells from controls and hypertensive patients in the absence of or during antihypertensive treatment. (**A**) GSR and GSS. (**B**) GCLC and GCLM. (**C**) Intracellular GPXs (GPX2 mRNA levels were divided/adjusted by 2 x 10^3 factor). Quantitative polymerase chain reaction **i**s described in the Material and Methods section. Results are expressed as mean ± SEM. The error bars are represented in all boxes, but in some cases, the size is too small to be displayed. *Statistically significant differences between controls and untreated hypertensive patients. ‡Statistically significant differences between untreated and treated hypertensive patients.

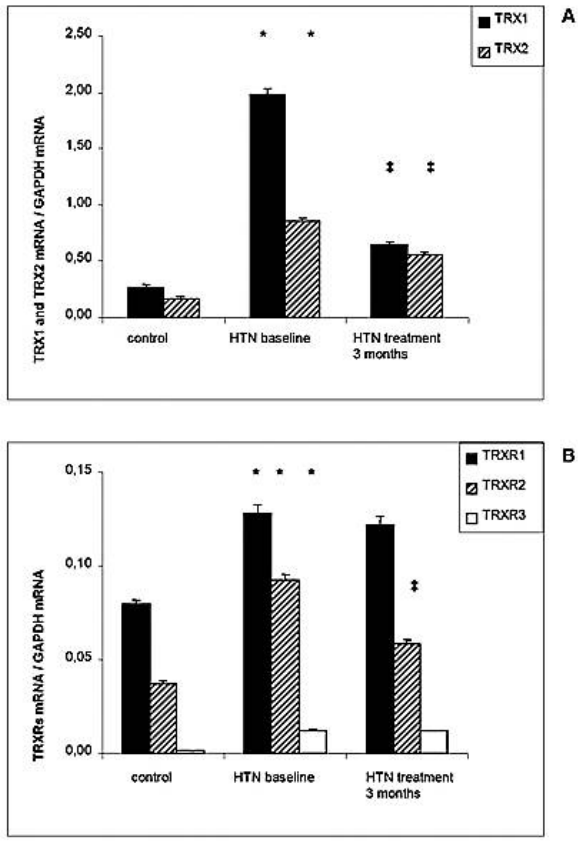

FIG. 2. mRNA levels of enzymes pertaining to the TRX system in mononuclear cells from controls and hypertensive patients in the absence of or during antihypertensive treatment. (**A**) TRX1 and TRX2. (**B**) TRXR1, TRXR2, and TRXR3. Quantitative polymerase chain reaction is described in the Material and Methods section. Results are expressed as mean ± SEM. The error bars are represented in all boxes, but in some cases, the size is too small to be displayed. *Statistically significant differences between controls and untreated hypertensive patients. ‡Statistically significant differences between untreated and treated hypertensive patients.

The present study tested the final active compounds (GSH and Trx) of these antioxidant systems in a human model of chronic OS such as essential hypertension, excluding other potent stimulators of OS: diabetes, dyslipidemia, or smoking. In untreated patients, no previous antihypertensive medication was allowed, to avoid not only the impact of BP reduction, but also the potential drug-induced changes in ROS generation or in the activity of antioxidant mechanisms in this group. We analyzed mRNA values in circulating mononuclear cells that contribute to the OS burden on the cardiovascular system. Assessing mRNA levels of genes involved in GSH or Trx systems allows us, overall, to know the mononuclear cells' response mediated by both systems to the chronic ROS overload.

KNOWLEDGE ABOUT BEHAVIOR OF BOTH SYSTEMS

Many times, the expression of both systems is enhanced when oxidative stress is increased (5, 8) but we found a profound disparity in hypertension that could be explained by a different regulation with the chronic OS present in hypertension. In this situation, little is known about the regulation and response of both systems. In nonchronic induced OS, GSH synthesis is induced by GSH conjugates, GSH depletion, and oxidative-stress molecules through activation of mRNA transcription of two GCL subunits, GCLC and GCLM (28). Although mRNA levels of both GCL subunits increased in response to OS, their ratio can differ depending on the stimulus

(9). Furthermore, GCL activity is coordinated with the activation of GSS to induce GSH synthesis. A direct relation has been observed between mRNA and protein levels in GCL subunits and GSS (9). In contrast, the regulation of GPXs and TxrRs, which are selenoproteins, have different paths of transcription, mRNA stabilization, and translation, but when the selenium levels are normal, the protein level strongly depends on mRNA levels (1).

DIFFERENT BEHAVIOR OF GSH AND TRX SYSTEMS

Significant differences in behavior of the two systems were reflected in response of the GSH system by reduced mRNA levels and increased levels of mRNA from the Trx system to the chronic OS present in hypertension. The differences in behavior were present not only in the basal situation of hypertensives, but also during antihypertensive treatment. Although in the GSH system, mRNA levels did not improve despite the reduction in OS, and move away from control levels, the mRNA levels of the Trx system, although decreasing, were still higher than those in control subjects. These data may indicate an activation of the Trx system by chronic increased OS levels and the persistence, but at lower levels, under antihypertensive treatment.

A limitation of the present study is that the relations among mRNA, protein level, and enzymatic activity are complex, and a direct correlation can not be assumed. Posttranslational regulation can modify the protein synthesis in several ways. Even in genes with negligible posttranslational regulation, protein levels and their activity may be influenced by the ROS impact, which produces molecular changes that both inactivate the protein and predispose it to rapid degradation (9, 28). The present study measured blood levels of GSH and Trx, and mRNA levels were assessed in mononuclear cells, assuming that the degree of mRNA levels in mononuclear cells is indicative of general response against chronic increased OS levels in hypertensive patients.

EFFECT OF TREATMENT ON OXIDATIVE STRESS AND ON MRNA LEVELS

Although the study was not designed to look for differences between different kinds of treatment, it is worth mentioning the absence of differences among the treatment groups. The absence of differences in mRNA levels was concordant with the observation that lowering blood pressure and OS are independent of the kind of drug used (2). Although drugs affecting angiotensin II levels or activity are more specific to blocking the ROS generation by the NADPH-oxidase system (6), other mechanisms are responsible for ROS generation in hypertension. In this way, the increased levels of OS and the lowered levels of mRNA from the GSH system can indicate that mononuclear cells from hypertensive patients have a reduced protection against OS. In addition, patients under treatment show a weaker potential protection, and this can facilitate OS injury by sporadic or continuous OS insults. These alterations may facilitate development of hypertension, endothelial dysfunction, and target-organ damage (12, 17, 27, 30).

The potential role of antioxidant treatment to reduce hypertension-induced cardiovascular risk is a matter of debate. Long-term clinical trials failed to observe a beneficial impact of vitamins E or C in reducing cardiovascular events of high-risk patients, despite improvement in endothelial dysfunction and other surrogate markers of atherosclerosis (26). The results are positive in reducing blood pressure levels and renal damage in animal studies (24). Both vitamins E and C reduce OS, acting as ROS scavengers. The possibility of therapies inducing improvement in the mechanisms controlling the GSH and Trx systems must be explored in the future. *N*-acetylcysteine and *S*-adenosyl-L-methionine can increase levels of GSH (23); however, no information exists about the impact these antioxidants on the genetic regulation of the GSH and Trx systems in human hypertension.

TRX SYSTEM IS NOT ABLE TO CONTROL INCREASED OXIDATIVE STRESS LEVELS

Finally, these data also offer information about the relative importance of the GSH and the Trx systems in the defense against chronic ROS overproduction in hypertension. According to the observed behavior and the OS status, the GSH system plays a more major role than does the Trx system in hypertension in OS control. Probably the Trx system genes are induced when the intracellular GSH is consumed by too much hypertension-induced ROS. Therefore, the induction of the Trx system is an alarm in the host defense against OS. Induced Trx itself, however, is not enough to scavenge ROS generated by hypertensive stress but can promote the transcription of other antioxidant enzymes by its redox regulation of transcriptional factors. Despite this, the Trx system is not able to control the increased ROS generation when an inadequate response by the GSH system exists. Whether the different behaviors of the two systems can be observed in other human models of human chronic OS is an intriguing question.

CONCLUSIONS

In conclusion, the differences in the mRNA levels of both the GSH and Trx systems show a disparity in the response of each one, an inadequate response by the GSH system and a normal one by the Trx system. Despite Trx system activation, it seems to be insufficient for maintaining a normal redox status in hypertensive subjects. The inappropriate response of the GSH system and the insufficient control of OS by the Trx system despite its overactivity, leave hypertensive patients highly exposed to the increased ROS generation and its consequences.

ABBREVIATIONS

8-Oxo-dG, 8-oxo-deoxiguanosin; BP, blood pressure; GCL, glutamate cysteine ligase; GSH, glutathione; GSSG, glutathione disulfide or oxidized; GPX, glutathione peroxi-

dase; GSR, Glutathione reductase; GSS, glutathione synthetase; MDA, malondialdehide; OS, oxidative stress; ROS, reactive oxygen species; Trx, thioredoxin; TrxR, thioredoxin reductase.

APPENDIX

Notes

1. Materials and methods

The Sandwich enzyme-linked immunosorbent assay (ELISA) kit for Trx1 was provided by Redox Bio Science (Kyoto, Japan). Ready-To-Go You Prime First-Strand Beads Kit was purchased from GE Healthcare Bio-Sciences (Uppsala, Sweden). Primers were from Sigma-Aldrich (St. Louis, MO).

2. Selection of study participants

Patients were invited to participate if the following criteria were met: (a) essential hypertension defined according to the criteria of the VII Joint National Committee (4); (b) aged 25–55 years; (c) WHO grade I–II; and (d) never previously treated for hypertension. Patients with diabetes mellitus, fasting glucose in serum >120 mg/dl, total cholesterol levels >240 mg/dl, or cigarette consumption were excluded. A group of healthy, normotensive, nonsmokers were selected as a control group. The Ethics Committee of the Hospital approved the study, and the patients gave their informed written consent to participate.

After enrollment, hypertensive patients were given the usual treatment, which included a nonpharmacologic treatment consisting of moderate salt restriction and a low-calorie diet, if overweight, with or without a course of nonrandomly allocated antihypertensive drugs based on β-blockers (atenolol), or angiotensin-receptor blocker (telmisartan). Hydrochlorothiazide was added when necessary to maintain the blood pressure (BP) goal of <135/85 mm Hg.

Blood pressure was measured using a mercury sphygmomanometer in the office, according to the recommendations of the British Hypertension Society (19), and using an oscillometric monitor (Spacelabs 90202 or 90207, Issaguah, WA) in ambulatory conditions during 24 h on a regular workday. Blood samples were obtained in the morning after a minimum of 8 h of fasting. Serum biochemical profiles were measured using an autoanalyzer and under standard procedures.

3. GSH, GSSG, Trx, MDA, and 8-oxo-dG measurement

The OS status was evaluated by measuring the GSSG/GSH ratio in plasma and mononuclear cells, as well as the ROS-induced by-products, malondialdehide (MDA) and 8-oxo-deoxiguanosin (8-oxo-dG). GSH and Trx levels were measured to assess the functional capacity of the two systems.

Heparinized whole blood was diluted with saline medium, and mononuclear cells were isolated by Ficoll-Hypaque centrifugation followed by three washes. Blood plasma was separated by whole-blood centrifugation. MDA, GSH, and GSSG levels were measured in plasma and in mononuclear cells using previously described protocols based on HPLC (2, 16, 29). The Trx levels were measured in plasma using a sensitive sandwich ELISA kit (Redox Bio Science, Kyoto, Japan) according to the manufacturer's instructions. The cellular protein content was measured using the Bradford method.

For genomic 8-oxo-dG quantification, DNA was isolated by following the method of Gupta with the modification described by Muñiz (14). Isolated DNA was washed twice with 70% ethanol, dried, and dissolved in 200 µl of TE buffer for its enzymatic digestion and was analyzed as previously described (5).

4. Total RNA extraction and quantitative polymerase chain reaction (Q-PCR)

The response to chronic OS was assessed by measuring the mRNA levels of the components of the GSH and Trx systems in mononuclear cells. Total RNA was extracted from blood mononuclear cells purified by the Ficoll Hypaque method, and RNA was treated with DNase I from Roche Diagnostics (Basel, Switzerland) to remove any contaminating DNA (24). One microgram of treated RNA was reverse-transcribed to cDNA using Ready-To-Go You Prime First-Strand Beads (Amersham Pharmacia Biotech) with random primers. Primer sets from each candidate gene were designed by Primer3 software (22) and synthesized by Sigma (Table 3). Measurement of gene expression was performed using the ABI PRISM 7000 Sequence Detection System and SYBR Green Master Mix (Applied Biosystems; Madrid, Spain) by following the manufacturer's instructions. Standard curves were generated for each gene to determine the PCR efficiency and quantification of the copies of each mRNA in the samples. Linear regression analysis of all standard curves documented in all cases an R^2 value of ≥ 0.99. Normalization of samples was performed by the reference gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as previously described (26). All PCR products were tested with agarose gel electrophoresis and with dissociation curves (Fig. 3). Correct amplification was verified in all samples and in all reactions by dissociation curves.

5. Statistical analysis

For each variable, the values were expressed as mean ± standard error of the mean values. The differences between hypertensive and control groups were assessed using a nonparametric unpaired test and a paired test to assess the differences between baseline and during treatment in hypertensive patients. Two-tailed values of $p < 0.05$ were considered statistically significant.

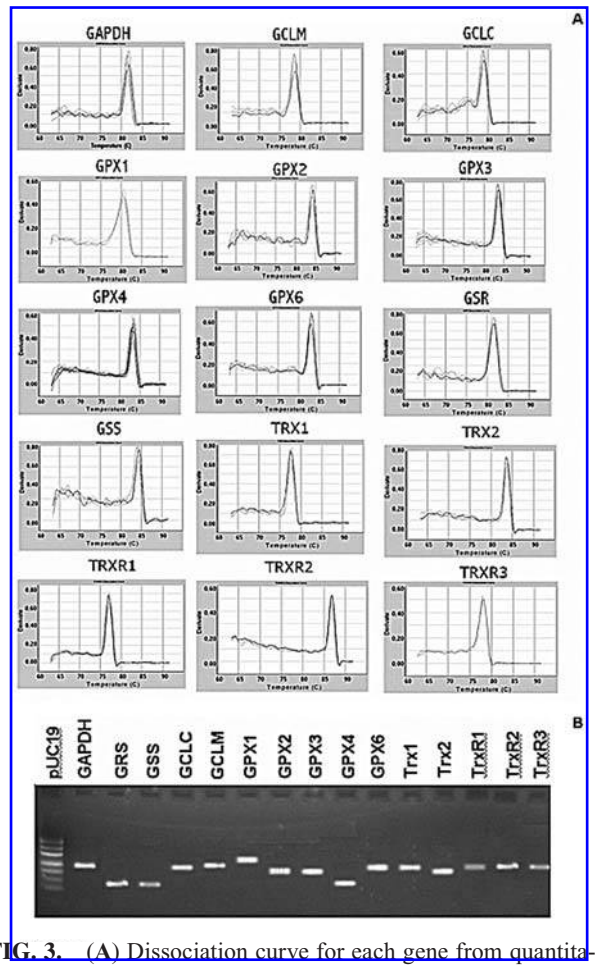

FIG. 3. (**A**) Dissociation curve for each gene from quantitative PCR, and (**B**) agarose gel electrophoresis gel photograph of PCR products. Quantitative polymerase chain reaction **i**s described in the Material and Methods section.

TABLE 3. QUANTITATIVE RT-PCRA PRIMERS

Symbol	*Gene product*	*Accession number*	*Sequence*
GAPDH	Glyceraldehyde-3 phosphate dehydrogenase	NM_002046	(+)-CTGGAAGATGGTGATGGGATTTC (−)-GAAGGTGAAGGTCGGAGTCAAC
GSR	Glutathione reductase	NM_000637	(+)-ATCCCCGGTGCCAGCTTAGG (−)-AGCAATGTAACCTGCACCAACAA
GSS	Glutathione synthetase	NM_000178	(+)CTCACTGGGATGTGGGTGAAGAAG (−)-TCCTCCCCATATAGGTTGTTACCTC
GCLC	Glutamate-cysteine ligase, catalytic subunit	NM_001498	(+)-GCGATGAGGTGGAATACATGTTG (−)-CTCCCATACTCTGGTCTCCAAAGG
GCLM	Glutamate-cysteine ligase, modifier subunit	NM_002061	(+)-AATCCTGATGAAAGAGAAGAAATGAAAG (−)-TCCATCTTCAATAGGAGGTGAAGC
GPX1	Cellular glutathione peroxidase	NM_000581	(+)-AAACCAGTTTGGGCATCAGGAGAAC (−)-ATGAGCTTGGGGTCGGTCATAAG
GPX2	Glutathione peroxidase 2 (gastrointestinal)	NM_002083	(+)-CATCAGGAGAACTGTCAGAATGAGG (−)-TAGGGGAGCTTGTCCTTCAGGTAG
GPX3	Glutathione peroxidase 3 (plasma)	NM_002084	(+)-CAGAGCCGGGGACAAGAGAAG (−)-CGGACATACTTGAGGGTAGGAAGGA
GPX4	Glutathione peroxidase 4 (phospholipid hydroperoxidase)	NM_002085	(+)-AGATCCACGAATGTCCCAAGTC (−)-ACGTTGGTGACGATGCACAC
GPX6	Glutathione peroxidase 6 (olfactory)	NM_182701	(+)-GGGGTAACAGGCACCATCTATGAG (−)-TTCAGCTCCTCCTGTAGTGCATTC
TRXR1	Thioredoxin reductase 1	NM_003330	(+)-CCCTGCAAGACTCTCGAAATTATG (−)-CTACTCGGTAGCCCCAATTCAAAG
TRXR2	Thioredoxin reductase 2	NM_145748	(+)-TATGGCTGTGTGGGGCTGTC (−)-ACCATCTTTACATAACACTGGGATGC
TRXR3	Thioredoxin reductase 3	BC050032	(+)-TGGTGGCACTTGTGTAAATGTAGG (−)-GGTTCTGAATCGCTTTTGTCATTG
TRX1	Thioredoxin	NM_003329	(+)-AGACTCCAGCAGCCAAGATGG (−)-GAGAGGGAATGAAAGAAAGGCTTG
TRX2	Mitochondrial thioredoxin	NM_012473	(+)-CACTTCCAGAGCCCTGCAGAC (−)-CACTGGTGTCTCACTGTTGACCAC

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REFERENCES

1. Bermano G, Arthur JR, and Hesketh JE. Selective control of cytosolic glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase mRNA stability by selenium supply. *FEBS Lett* 387: 157–160, 1996.
2. Chaves FJ, Mansego ML, Blesa S, Redon J, Tormo MC, Espinosa O, Gonzalez-Albert V, and Saez G. Mechanisms implicated in the oxidative stress of hypertension. *Am J Hypertens* (in press).
3. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jones DW, Materson BJ, Oparil S, Wright JT, Roccella EJ, and Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program Coordinating Committee. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 42: 1206–1252, 2003.
4. Haendeler J, Tischler V, Hoffmann J, Zeiher AM, and Dimmeler S. Low doses of reactive oxygen species protect endothelial cells from apoptosis by increasing thioredoxin-1 expression. *FEBS Lett* 577: 427–433, 2004.
5. Hagg D, Englund MC, Jernas M, Schmidt C, Wiklund O, Hulten LM, Ohlsson BG, Carlsson LM, Carlsson B, and Svensson PA. Oxidized LDL induces a coordinated up-regulation of the glutathione and thioredoxin systems in human macrophages. *Atherosclerosis* 185: 282–289, 2006.
6. Hirota K, Nakamura H, Masutani H, and Yodoi J. Thioredoxin superfamily and thioredoxin-inducing agents. *Ann N Y Acad Sci* 957: 189–199, 2002.
7. Holmgren A. Antioxidant functions of thioredoxin and glutaredoxin systems. *Antioxid Redox Signal* 2: 811–820, 2000.
8. Jurado J, Prieto-Alamo MJ, Madrid-Risquez J, and Pueyo C. Absolute gene expression patterns of thioredoxin and glutaredoxin redox systems in mouse. *J Biol Chem* 278: 45546–45554, 2003.
9. Krzywanski DM, Dickinson DA, Iles KE, Wigley AF, Franklin CC, Liu RM, Kavanagh TJ, and Forman HJ. Variable regulation of glutamate cysteine ligase subunit proteins affects glutathione biosynthesis in response to oxidative stress. *Arch Biochem Biophys* 423: 116–125, 2004.
10. Matsuzaka Y, Okamoto K, Mabuchi T, Iizuka M, Ozawa A, Oka A, Tamiya G, Kulski JK, and Inoko H. Identification and characterization of novel variants of the thioredoxin reductase 3 new transcript 1 TXNRD3NT1. *Mamm Genome* 16: 41–49, 2005.
11. Miwa K, Kishimoto C, Nakamura H, Makita T, Ishii K, Okuda N, Yodoi J, and Sasayama S. Serum thioredoxin and alpha-tocopherol concentrations in patients with major risk factors. *Circ J* 69: 291–294, 2005.
12. Modlinger PS, Wilcox CS, and Aslam S. Nitric oxide, oxidative stress, and progression of chronic renal failure. *Semin Nephrol* 24: 354–365, 2004.

13. Muñiz P, Valls V, Perez-Broseta C, Iradi A, Climent JV, Oliva MR, and Sáez GT. The role of 8-hydroxy-2-deoxyguanosine in rifamycin-induced DNA damage. *Free Radic Biol Med* 18: 747–755, 1995.
14. Navarro J, Obrador E, Pellicer JA, Asensi M, Viña J, and Estrela JM. Blood glutathione as an index of radiation-induced oxidative stress in mice and humans. *Free Radic Biol Med* 22: 1203–1209, 1997.
15. Nkabyo YS, Ziegler TR, Gu LH, Watson WH, and Jones DP. Glutathione and thioredoxin redox during differentiation in human colonic epithelial (Caco-2) cells. *Am J Physiol Gastrointest Liver Physiol* 283: G1352–G1359, 2002.
16. O´Brien E, Asmar R, Beilin L, Imai Y, Mallion JM, Mancia G, Mengden T, Myers M Padfiels P, Palatini P, Parati G, Pickering T, Redon J, Staessen J, Stergiou G, and Verdecchia P, on behalf of the European Society of Hypertension Working Group on Blood Pressure Monitoring. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *J Hypertens* 21: 821–848, 2003.
17. Raij L. Nitric oxide, salt sensitivity, and cardiorenal injury in hypertension. *Semin Nephrol* 19: 296–303, 1999.
18. Redon J, Oliva MR, Tormos C, Giner V, Chaves FJ, Iradi A, and Saez GT. Antioxidant activities and oxidative stress byproducts in human hypertension. *Hypertension* 41: 1096–1101, 2003.
19. Rozen S, Skaletsky HJ. Primer3 on the www for general users and for biologist programmers. In: eds. *Bioinformatics, Methods and Protocols: Methods in Molecular Biology*, edited by Krawetz S and Misener S. Humana Press, Totowa, NJ, 2000, pp. 365–286.
20. Saez GT, Tormos C, Giner V, Chaves FJ, Lozano JV, Iradi A, and Redon J. Factors related to the impact of antihypertensive treatment in antioxidant activities and oxidative stress byproducts in human hypertension. *Am J Hypertens* 17: 809–816, 2004.
21. Sies H. Glutathione and its role in cellular functions. *Free Radic Biol Med* 27: 916–921, 1999.
22. Tanito M, Nakamura H, Kwon YW, Teratani A, Masutani H, Shioji K, Kishimoto C, Ohira A, Horie R, and Yodoi J. Enhanced oxidative stress and impaired thioredoxin expression in spontaneously hypertensive rats. *Antioxid Redox Signal* 46: 89–97, 2004.
23. Terneus MV, Kiningham KK, Carpenter AB, Sullivan SB, and Valentovic MA. Comparison of S-Adenosyl-L-methionine and N-acetylcysteine protective effects on acetaminophen hepatic toxicity. *J Pharmacol Exp Ther* 2006 (in press, accessible by PubMed).
24. Tian N, Thrasher KD, Gundy PD, Hughson MD, and Manning RD Jr. Antioxidant treatment prevents renal damage and dysfunction and reduces arterial pressure in salt-sensitive hypertension. *Hypertension* 45: 934–939, 2005.
25. Vaziri ND, Wang XQ, Oveisi F, and Rad B. Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. *Hypertension* 36: 142–146, 2000.
26. Wassmann S, Wassmann K, and Nickenig G. Modulation of oxidant and antioxidant enzyme expression and function in vascular cells. *Hypertension* 44: 381–386, 2004.
27. Wilcox CS. Oxidative stress and nitric oxide deficiency in the kidney: a critical link to hypertension? *Am J Physiol Regul Integr Comp Physiol* 289:913–935, 2005.
28. Wild AC and Mulcahy RT. Regulation of gamma-glutamylcysteine synthetase subunit gene expression: insights into transcriptional control of antioxidant defenses. *Free Radic Res* 32: 281–301, 2000.
29. Wong SHY, Knight JA, Hopfer SM, Zaharia O. Leach CN, and Sunderman FW. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clin Chem* 33: 214–220, 1987.
30. Zalba G, Jose GS, Moreno MU, Fortuno MA, Fortuno A, Beaumont FJ, and Diez J. Oxidative stress in arterial hypertension: role of NAD(P)H oxidase. *Hypertension* 38: 1395–1399, 2001.

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