

# THE ROLE OF REACTIVE OXYGEN SPECIES IN RENAL HYPERTENSION

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## REZUMAT

Lucrarea de față face o trecere în revistă a implicării speciilor reactive de oxigen (ROS) în multiplele verigi patologice care determină disfuncție endotelială, disfuncție renală și leziuni renale la pacienții hipertensivi și cu afectare structurală renală. Sursele de ROS în bolile renale sunt reprezentate de celulele inflamatorii (neutrofilele și macrofagele), celulele tubulare, mezangiale, fibroblaștii, celulele endoteliale, și de sistemul renină-angiotensină, și pot determina în exces creșterea valorilor tensionale prin mai multe mecanisme (disfuncție endotelială, perturbarea mecanismelor de feedback tubulo-glomerular, cu creșterea retenției hidrosaline, apoptoză celulară și fibroză renală, care conduc la stadii avansate de boală renală și hipertensiune dependentă de volum). Totodată ROS au rol în remodelarea vasculară și ateroscleroza la pacienții hipertensivi, cu cât stadiul insuficienței renale cronice este mai avansat. Sunt abordate separat rolul ROS în principalele tipuri de hipertensiune arterială de etiologie renală (HTA renovasculară, din nefropatia diabetică, glomerulonefrite, afecțiuni tubulo-interstițiale renale, insuficiență renală cronică). În acest context se conturează noi perspective terapeutice ale HTA privind utilizarea medicamentelor antioxidante (vitamina E, substanțe SOD-mimetice), antiinflamatorii (mycofenolat mofetil) și a terapiei combinate - IECA și antagoniști de receptori de angiotensină II - în diminuarea atât a valorilor tensionale, cât și a afectării renale.

**Cuvinte cheie:** specii reactive de oxigen, hipertensiune arterială, stres oxidativ, disfuncție endotelială, sistem antioxidant

## ABSTRACT

This article summarizes of the implication of oxygen reactive species (ROS) in the multiple pathological links determining endothelial dysfunction, renal dysfunction and renal lesions at hypertensive patients and with renal structural affection. Sources of ROS in the renal diseases are represented by the inflammatory cells (neutrophils and macrophage), tubular, mesangial, fibroblast cells, endothelial cells and by the renin-angiotensin system (RAS) and they can determine in excess the raise of tensional values through more mechanisms (endothelial dysfunction, tubuloglomerular feedback mechanisms perturbation with the increase of salt and water retention, cell apoptosis and renal fibrosis leading to advanced stages of renal disease and hypertension depending on the volume). ROS have as well a role in vascular remodelling and atherosclerosis at hypertensive patients as much as the stage of chronic renal failure is more advanced. The role of ROS in the main types of hypertension of renal etiology (renovascular hypertension, diabetic nephropathy, glomerulonephritides, renal tubulointerstitial diseases, end-stage renal disease) is separately approached. Within this context there are new therapeutic perspectives of renal hypertension taking place regarding the usage of antioxidant drugs (vitamin E, superoxide dismutase-mimetic substances), anti-inflammatory (mycophenolate mofetil) and combined therapy - angiotensin-converting inhibitors (ACEI) and angiotensin-II receptor blocker (ARB) - in diminishing the tension values as well as renal damage.

**Key words:** reactive oxygen species, arterial hypertension oxidative stress, endothelial dysfunction, antioxidant defence system

## INTRODUCTION

Arterial hypertension (HTA) is a common feature in the renal diseases. Even in 1% of patients with essential arterial hypertension there is a degree of renal involvement. Experimental studies have been demonstrated that in essential hypertension exists microvascular and tubulointerstitial injury.<sup>1</sup> Thus, any

factor determining HTA for a relatively short period of time determines perpetuating renal subclinic lesions and maintains the raised tension values even after the initiating factor has been removed.

Studies on spontaneously hypertensive rats (SHR) have found that first changes that appears are renal interstitial inflammatory modifications and are followed by raised blood pressure values that further worsen the local renal inflammatory process.<sup>2</sup>

The tension values are correlated to the degree of endothelial dysfunction, of renal dysfunction, with those of renal lesions and it seems that the link between all these factors determining the disease severity and prognosis is represented by the absolute value of oxidative stress. The oxidative stress is a relatively new notion and it appears that any external action on the body finally determines the increase of oxidative

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stress and the severity of the disease. The oxidative stress is equivalent to the breeding of oxygen reactive species (ROS). It has been found that oxidative stress is increased in HTA, the renal involvement amplifies it and it has an additional effect to the cardiovascular risk factors. There are today sufficient researches corresponding to clinical notices regarding the severity of cardiovascular injury and the rate of progression in renal dysfunction at the hypertensive patients with at least one factor of risk, equating the raise of oxidative stress. The more the oxidative stress is greater the tension values are more difficult to control and the cardiovascular injury more serious in renovascular hypertension, hypertension of diabetic nephropathy, HTA of renal inflammatory lesions comparatively to essential HTA.

The oxidative stress seems to be an equivalent of the plurality of risk factors in producing major cardiovascular events in hypertensions of different causes.

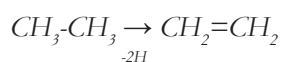
The mechanisms of production the reactive oxygen species are different in part, as free radicals of oxygen but they all finally lead at renal damage. It also explains the different evolution to end-stage renal disease in case of patients with arterial hypertension. Thus, in essential arterial hypertension, nephroangiosclerosis appears after an evolution of approximately 20 of years of disease. If there is added the supplementary risk factors the evolution of the renal disease is accelerated. This evolution is directly proportional whit severity of the oxidative stress organism is suffering from.

## **PATHOPHYSIOLOGICAL FUNDAMENTALS**

### **ROS and the antioxidant defense system**

The reactive oxygen species (ROS) are substances with a high oxidation potential.

Classically, oxidation is considered to be the addition of oxygen to a composite or the hydrogen taking out not depending on the fact that oxygen enters or not in the respective composite, and reduction is considered as the inverted addition process of hydrogen.



The actual concepts define oxidation as the process electrons are lost through and reduction as electrons receipt. In a reaction, a substance receives electrons and the other cedes so we are talking here about an

oxide-reduction reaction.<sup>3,4</sup> ROS means any species capable to exist independently containing one or more electrons but in odd number in their outer orbits (like those occupying an atomic or molecular orbit). The reactive species of oxygen constitute a large class of chemical structures which also include the free oxygen radicals. They form by loosing a single electron from a non-radical or by receiving an electron on a non-radical. It is the easiest formed by breaking a covalent bond, through which each electron of the electron pair of the covalent bond remains on an atom. This mechanism is called homolytic fission. Within the water through this mechanism there is generated one of the most reactive species the hydroxyl radical OH•. The burning breaking covalent bond of C-C, C-H and C-O is a process of generating free radicals. There is also a heterolytic fission where after the break of a covalent bond, an atom receives both electrons, which means that it has a negative load while the other is positively loaded.

There have been largely studied: hydroxyl radicals (•OH), superoxide (O<sub>2</sub>•<sup>-</sup>), nitric oxide (•NO), singlet oxygen (<sup>1</sup>O<sub>2</sub>), ozone (O<sub>3</sub>), thiyl radicals (RS•), radicals of carbon atom (•CC<sub>13</sub>), (•O<sub>2</sub>CC<sub>13</sub>), peroxyxynitrite radical (OONO•).

The increased oxidative potential is also detained by the nitrogen reactive species. They result through the reaction between NO and the superoxide radical (O<sub>2</sub>•<sup>-</sup>) and form peroxyxynitrite (ONOO•).

A major importance in the oxidoreduction processes of the organism is also detained by Fe as catalyzer of the reactions producing radicals. Fe is incriminated in inducing renal lesions. The iron is a strong prooxidant capable to induce lesions and the death of tubular epithelial cells and of those vascular endothelial.<sup>5</sup>

Man cannot live without oxygen. But there has been proved that in animals and plants also a too large quantity of oxygen is toxic. Breathing air rich in oxygen determines acute toxicity of the central nervous system, besides other symptoms. ROS the same who in physiological doses have beneficial effects in organism in high concentrations are toxic and determine lesions at a cell level until molecular level. There actions against ROS the antioxidant defense system, reacting with or inactivating ROS. They are represented by GSH/GSSG system, the reduced/oxidant cysteine system, pyruvate/lactate system, pyridine nucleotide bases. The antioxidant defense system also contained antioxidant enzymes: superoxide dismutase (SOD), catalase and glutathione peroxidase whose down adjustment determines oxidative stress.<sup>6</sup> Catalase and

SOD transforms the superoxide ion in oxygenated water ( $H_2O_2$ ). At its turn, it can generate hydroxyl radicals.

The first interfering in defending against ROS is the glutathione GSH/GSSG system. Its plasmatic and intracellular level increases at administering ascorbic acid.<sup>7</sup> In the diseases oxidative stress is higher, it has reduced plasmatic and intracellular levels.

The reduced/oxidized cysteine system has an increased redox potential. The cysteine contains a thiol group which can exist in four different forms as oxidised: R-S-OH or R-S-S-R ; S-nitrosylated: R-S-NO; glutathionylated: R-S-S-G, reduced R-SH.<sup>8</sup>

### The physiological role of ROS

ROS in small doses have a physiological role in the body, functioning as adjusters of cellular responses. They are implied in activating different intracellular signalling ways. Among the ROS the following have an important role in the signal transduction and transcription: superoxide  $O_2\bullet$  and nitrogen  $NO\bullet$ .<sup>9</sup>

There are three general possibilities to influence these ways of intracellular transmission of the signal. First, by connecting to the membranar receptor, there is produced its conformational modification, usually a dimerization leading to transitory liberating of  $H_2O_2$  with activation of the intracellular signaling way. Thus ROS activates the following transmission ways of intracellular signal the proteine membranar way G, Ras, Rho si Rac-1. ROS also have a second messenger role especially on intracellular ways responding on cellular proliferation and hyperplasia that is cellular increase. Thus they participate to activating Src kinases (or Akt) and ERK1/2 of the mitogen-activated protein kinases (MAPK).<sup>10</sup> Thirdly, they can operate at the level or next to the nucleus determining the activation of NF-kB with a role in genic transcription. NF-kB is implied in genic expressing of adhesion molecules, cytokines, chemokines and acute phase proteins. Many of these ways have an adjustment stage implying ROS. Thus, there is also explained the ROS role in determining hyperplasia and hypertrophy FMN and in vascular remodelling but also in the initiation and maintenance of the immune process at the level of the mesangial cell and renal interstitium. It seems that  $H_2O_2$  imitates the effect of insulin on glucose transport and of lipid synthesis in the rats adipocytes.<sup>11</sup>

Antibodies have the capacity to catalyze water oxidation at free radicals producing by this the destruction of the pathogen agent. At the level of oxidative lesions produced by antibodies on the cellular membrane, cells expose phosphatidylserine and are phagocyted.

### The pathological role of ROS

The reactive oxygen species represent the major mediators of cellular injury, and their increased production, the perturbation of the antioxidant balance and the lipid peroxidation induction represent the main mechanism in many types of diseases, including the renal, cardiovascular diseases, cancer and the aging process.

The excessively free radicals will attack groups contained in tissular structures. Thus proteins containing amino acids (AA) with thiol groups as metionin and cistein will be oxidized at this level; will form disulphuric bridges modifying either the structure or the respective enzyme activity. It could influence the proteins packing in endoplasmatic reticulum.

Another AA which can be oxidized is tyrosine contained in tyrosine kinases. When the signal transmission goes for phosphorylation of proteins, the redox modelling becomes very important for dephosphorylation of proteins. The proteic chains connected through a dityrosine connection are irreversible and considered proteins with an advanced degree of oxidation.

Another oxidation mechanism of proteins is the one forming carbonyl groups  $-C=O$  with generating aldehydes and ketones. They are produced when the oxidative stress is very high, as for example in: hyperglycaemic coma or in chronic renal failure. The products formed like that are considered proteins with advanced degree of oxidation.

The free oxygen radicals are formed on the way of lipids peroxidation. In diabetes mellitus there are formed proteins with a high degree of glycosylation being as well the result of a process of carbonic groups being in the same time the result of a reduction process of carbonyl groups with the groups of AA of proteins.

The markers of the oxidative stress increase are considered: 3-chlorotyrosine, 3-nitrotyrosine and dityrosine, malonyldialdehyde, acrolein (2-propenal), 4-hydroxy-2-nonenal, oxidised LDL-c, 8-hydroxi-2-deoxyguanosine.

ROS have a role in the renal inflammatory processes. ROS issued in the interstitium produce modifications conformational to membranar proteins and through it they acquire immunogenic potential. It is the case of decarboxylase oxidation of glutamic acid determining the generation of immunogenic epitopes and finally diabetes mellitus. The hydroxyl radicals can interact with chromatin and produce alterations of nucleosomes. Thus, in lupus eritematosus 8-hydroxi-guanozin is detected in immune complexes.

ROS are also involved in cellular apoptosis. The excessive production has a role in the action of TNF on the cells. The cells are compartmented and ROS has no free access cellular components. The superoxide anion cannot pass over the cellular membrane, but in the case of SOD attachment of heparin sulphate glycosaminoglycans to endothelial cell it is transformed in  $H_2O_2$  and thus the oxidative stress can also propagate within the cell. If the intracellular defence mechanism is surpassed, first, the GSH decreases. In this case, the free radicals will attack other intracellular proteins determining the precipitation in cytosol and their intracellular accumulation the proteolytic system is also affected. The free radicals will also attack intracellular proteins with enzymatic role modifying their activity. In cases of increased accumulation of intracellular ROS, when the antioxidant defence mechanisms are surpassed, the mitochondrial chain by electronic transfer will be injured and cytochrome C is lost from the interior of mitochondria, a first step to cellular apoptosis but still reversible. The moment of irreversibility of modifications is constituted by the mitochondrial membrane loss, where oxidation Apaf-1 leads to "caspases" activation. Thus, free radicals will be discharged in cytoplasm, the phosphatidylserine oxidation is produced, their externalization on cellular membrane and the recognition of the cells by macrophage for clearance.<sup>6</sup>

### **The relationship renin-angiotensin system (RAS)- reactive oxygen species (ROS) in renal hypertension**

RAS has a major role in producing arterial hypertension of renal diseases but also in the appearance of renal lesions.

Ag II acting on the receptors AT1 of smooth muscular fiber activates NAD(P)H-oxidase and determines the increasing issuance of intracellular ROS and intense vasoconstriction. NAD(P)H-oxidase catalyzes the reduction with an electron of the oxygen molecule using NAD(P)H-oxidase as electron donor. Intracellular ROS will activate signaling ways implied in cellular hypertrophy and hyperplasia. Concomitantly NO local product interacts with superoxide radical forming peroxy-nitrate with role in lipidic peroxidation. There is produced a decrease of local NO which does not modulate induced vasoconstriction of Ag II. It would explain intense vasoconstriction of the renal diseases at the level of afferent and efferent arteriole with decrease of renal blood flow and of the glomerular filtration rate. In the inflammatory affections from the level of the kidney, although

existing a raised production of NO, it cannot exercise the renoprotective effect, rapidly combining with the superoxid anion and determining renal injury.

Through these actions SRA is implied in essential HTA, the tubulointerstitial nephropathies, in glomerulonephritides and in renovascular hypertension. In the renal inflammatory affections there is an excessive supplementary activation of local RAS which does not respond to the physiological action of systemic RAS. The cells have an increased expression of Ag II receptors without also meaning the decrease of Ag II activity.

There has been demonstrated in experimental studies that the perfusion with Ag II induces leucocitary margination. Ag II operating on the neutrophils determines the increase of ROS production intra and extracellular by translocating NAD(P)H-oxidase on cellular membranes, with phosphorylation and rapid activation of ERK1/2, JNK1/2 and p38MAPK and activation of NF-Kb. The activation NF-kB increases the expression of adhesion molecules on the neutrophil surface, the issuance of cytokine and chemotaxic factors.

The leucocytes adherent to the cell wall and the FMN also are the source of ROS and lead to vasoconstriction and vascular increase.

In tubulointerstitial inflammatory affections, Ag II excessively produced contributes to renal fibrosis. The activation of AT1 and AT2 receptors in proximal tubular cells break out a series of reactions as increase of transcription and synthesis TGF  $\beta$  and the inhibitor of kinases 2cyclic dependent (p27kip1) leading to hypertrophy of proximal tubular cells and increase the collagen synthesis of IV type [12]. It is added to this the synthesis of collagen type I and III of fibroblasts of basal membranes. Hypotesis, as the effect Ag II is mediated by ROS it is also sustained by experimental data. Thus, perfusion of Ag II determined intense vasoconstriction, which is diminished by ascorbic acid and mimetic SOD substances.

### **The endothelial dysfunction and proteinuria**

Endothelial dysfunction means the incapacity of the endothelium to make a proper autocrine and paracrine synthesis with perturbation of vasomotor and platelet antiagregants function. There has been noticed that at the hypertensive patients the serious endothelial dysfunction is associated to the proteinuria.<sup>13</sup> There are many pathological linkages, at different cellular and molecular types, mutually interconditioning and explains this association in: essential HTA, diabetic nephropathy, nephrotic syndrome. The proteinuria

- equivalent of renal injury and surrogate marker in following the evolution of the renal disease, is constituted through itself as inducing and aggravating element of renal damage.

The urinary albumin is taken over by tubular cells where activates the protein kinase C and determines the issuance of ROS. ROS activates NF- $\kappa$ B determining issuance of chemokines Rantes and MCP-1. They are excreted at the level of the basolateral membrane and thus attracting macrophages and initiating interstitial fibrosis.<sup>6</sup> A source of ROS is also represented by NAD(P)H-oxidase at the level of the cellular membrane. Thus, the urinary albumin is oxidized by forming carbonyl groups and thus transports oxidized lipids. The fat acids are connected to albumin after a previous auto-oxidation, reacting with the carbonyl groups. Thus, there is broken out the inflammatory cascade. In nephrotic syndrome the systemic oxidative stress is increased.

In general, antihypertensive drugs improve endothelial dysfunction and proteinuria. However, there is no correlation between tension values reduction and vasodilatation increase dependent on endothelium or/and of proteinuria. In some studies, the blockers of the calcium channels, betablockers or diuretics do not improve endothelial dysfunction to patients with essential hypertension, although all of these drugs have hypotensive effects.<sup>14</sup> It seems that endothelial dysfunction is improved at the decrease of blood pressure but this is not the directly determinant factor. And this factor seems to be oxidative stress. Similarly, only some classes of drugs, and even some drugs of the same class significantly reduce proteinuria. This particularity of the drug seems to owe to the antioxidant properties. As a consequence of the researches in the last years, there has been delineated the hypothesis that the nephroprotective and cardioprotective effect of the drug which inhibiting the renin-angiotensin system, as angiotensin-converting inhibitors and the angiotensin II - receptor blocker, are owed to the reduction of oxygen free radicals production.<sup>15</sup>

## **RENAL HYPERTENSION**

### **Hypertension of diabetic nephropathy**

HTA is presented in the first 7-10 years from its beginning at the patients with type 1 diabetes mellitus and over 40% of the patients with type II diabetes mellitus in the moment of the diagnosis, as a consequence of the disease or as preexistent HTA. Diabetic nephropathy is a long-term complication that occurs in 30-40% of patients with diabetes

mellitus.<sup>16</sup> Overt diabetic nephropathy is preceded by incipient stages without altering the renal function and with microalbuminuria (< 300 mg/24h), which signals an increased risk of progression to overt nephropathy (persistent proteinuria > 0.5 g/24 h or macroalbuminuria > 300 mg/24h).<sup>17</sup> The hereby HTA at the beginning of diabetes mellitus can accelerate the evolution of the renal disease. The patients will present microalbuminuria, endothelial dysfunction and increased oxidative stress. There is created a vicious circle attending and aggravating the tension values as well as renal lesions. The increased levels of glycaemia determines glycosylation of cellular and plasmatic proteins with forming the advanced glycosylation end-products (AGEPs). They have an increased oxidative potential and produce tissular lesions. Hyperglycaemia will determine the activation of protein kinase C, of other cytokine and grow factors which will produces free radicals of oxygen responsible of renal lesions. AGEPs connects to surface receptors called RAGEs, generates ROS and activates NF- $\kappa$ B and the way RAS - dependent.<sup>6</sup> AGEPs will determine through the agency of RAGEs the release of IL2, interferon  $\gamma$ , IL-1 $\beta$ .

In the diabetes mellitus hyperglycaemia determines directly the increase of the peroxide production of hydrogen of the mesangial murine cells and lipidic peroxidation in the mesangial glomerular cells.

Hyperglycaemia can determine through the agency of oxidative stress the apoptosis of mesangial cells. AGEPs stimulate synthesis of collagen on protein kinase way C and of TGF $\beta$ . On the way of RAGE-ERK1/2, AGEPs determine the phenotypic transforming of epithelial tubular cells in myofibroblasts. On experimental models at animals, diabetes associated with overexpression RAGEs is accompanied by renal hypertrophy, glomerular hypertrophy, mesangial expansion and advanced glomerulosclerosis. NO modulates glomerulosclerosis and tubulointerstitial fibrosis of diabetic nephropathy through direct action on the synthesis and proteins deterioration of extracellular matrix.

In the diabetes mellitus the increase of oxidative stress induces renal dysfunction representing the major mechanism in producing renal nephropathy.

### **The oxidative stress in renovascular hypertension**

In renovascular hypertension, we have a raised production of oxygen free radicals. The increased systemic levels of Ag II are the main cause of the oxidative stress increase, determining endothelial dysfunction with the decrease of the production of functional NO.

The endothelial dysfunction in renovascular hypertension was evaluated through the administration of acetylcholine in the left brachial artery in perfusion and determining an endothelium-dependent vasodilator response, which was reduced. Although perfusion with Ag II, in experimental studies, did not influence significantly the blood pressure and induced a lower vasodilatation comparatively to the control group, the forearm blood flow raised and urinary excretion of nitrite and nitrate, which meant the raise of the production of NO. Concomitantly, the urinary excretion of 8-hydroxy-2-deoxyguanosine, increased oxidative stress marker, diminished.<sup>14</sup>

After the renal angioplasty the arterial tension suddenly decreases, but the oxidative stress accentuated through urinary eliminations of 8-hydroxy-2-deoxyguanosine is improved after a longer period of time. The coming to normal of blood pressure after renal angioplasty does not immediately improve endothelial dysfunction, which suggests that not only the tension values increase determines endothelial dysfunction but also the free oxygen radicals. Thus, the endothelial dysfunction is correlated with the increase of blood pressure, getting more accentuated as higher its value.

#### **Arterial hypertension of glomerulonephritis**

The glomerulonephritis represent one of the main hypertension causes at relatively young adults. It is characterized through an inflammatory infiltrate at the level of the glomeruli, the presence of antibodies, autoantibodies and immune circulating complexes determining a raised production of oxygen reactive species at a local and systemic level as well. ROS will determine cellular lesions which finally lead to cellular apoptosis. Due to inflammatory infiltrate of glomerulonephritis there are locally generated, intrarenally, vasoconstrictor substances determining sodium retention. ROS also annihilates the local benefic effect of NO thus raising the sensitivity to salt.

As a conclusion, in glomerulonephritis ROS determines endothelial dysfunction, renal dysfunction, induce and maintain inflammatory processes and finally interstitial fibrosis.

#### **Arterial hypertension of tubulointerstitial inflammatory diseases**

The percentage of the patients with HTA is more reduced than to those with chronic glomerulonephritis and HTA and the evolution to chronic renal failure there appear after a longer period

of evolution. It seems that the quantity of ROS at systemic level is more reduces, the impact on the adjustment feedback of sodium retention is more reduced in the initial stages of disease. Hypertension appears in more late stages when the renal lesions are more advanced and the fibrosis extended.

Recent researches showed that there existed a relationship between renal interstitial inflammation, ARS local activity and ROS generation in HTA pathogenesis and a relationship directly proportional between these features and HTA severity. There is to be asked if the renal interstitial inflammatory infiltrate is a consequence or the cause of HTA. On experimental models at SRH rats, there has been found that the inflammatory infiltrate is present before the TA values increase. While HTA is installed, this interstitial inflammatory infiltrate is accentuated. This interstitial inflammation might have a role in the pathogenesis and maintaining raised levels of blood pressure.

In tubulointerstitial affections, the first aim is constituted by renal tubular cells. They have a reduced capacity to fight against oxidative and toxic stress. The proximal tubular cells take over at the renal tubular membrane the glutamine, cysteine and glycine. They have a limited capacity of taking over the cysteine at the level of the margin in brush and of synthesis of GSH. The decrease of the cellular glutathion peroxidase (GPX), determines the accumulation of ROS and the oxidation of membranes lipids. The glucose-6-phosphate dehydrogenase (G-6-PD), is the major source of NADPH and thus reduces the oxidative stress by raising the concentration of GSH.<sup>18</sup> The GSH regeneration of GSSG is mediated by GR on the expense of NADPH, thus the decrease of GR and of the activity of G-6-PD leads to oxidative stress increase. The administration of vitamin E, catalase, desferoxamine improves the cell content in GSH, G-6-PD and GR. In case of oxidative stress or attack from toxic substances to protect tubular cells, they take over glutathion of the extracellular fluid through basolateral membranes, but this process is also limited. GSH adjusts sphingomyelinase which produces ceramide implied in apoptosis of cells mediated by TNF $\alpha$ .

In all renal inflammatory diseases, either they are tubulointerstitial or glomerular, we have a raised production of free radicals responsible for cellular lesions. The raise of ROS production induced by NAD(P)H-oxidase determines structural and functional modifications in renal tubular membrane.

These free radicals are also implied in the activation of intracellular ways inducing interstitial fibrosis. An important role in interstitial fibrosis comes

to the transforming growth factor  $\beta$  (TGF $\beta$ ).<sup>19,20</sup> ROS stimulates the issuance of TGF $\beta$ 1 by the renal tubular cells and the immuno-competent cells. TGF  $\beta$ 1 determines hypertrophy of proximal tubular cells and stimulates the synthesis of collagen type IV. Ag II also stimulates the synthesis of TGF $\beta$ 1 of the renal tubular cells.

It seems that Smurf2 is implied in adjusting the signalize way of TGF $\beta$ . The expression smurf2 is different in kidneys and liver. It explains the different effect on hepatic and renal fibrosis of some drugs with antifibrogenetic effect only on the liver.<sup>12</sup>

The role TGF $\beta$ 1 regarding renal lesions, apoptosis and interstitial fibrosis was better studied in oxalic renal lithiasis. Thus, in laboratory experiments there has been showed that the exposition of tubular cells at oxalates induces synthesis by those of TGF $\beta$ 1. TGF- $\beta$ 1 activates Rac1 GTP-asis, one of the four cytosolic subunits of NADPH-oxidase. By activating NAD(P)H-oxidase, TGF  $\beta$ 1 stimulates the production of ROS inducing lipid peroxidation and cytolysis. The hypothesis is sustained by the experimental laboratory researches where the ROS production of renal tubular cells exposed to oxalates is inhibited by neutralized antibodies anti-TGF $\beta$ 1, antioxidant agents (vitamin E) and by inhibitors NAD(P)H-oxidase (diphenyliod). As well, the administration of antibodies anti TGF $\beta$ 1 has been shoed to suppress in vivo the activity TGF $\beta$ 1 and protects against renal fibrosis. The oxalates induce renal calculosis through the agency of ROS and lipid peroxidation. The lipid peroxidation precedes cytolysis induced by H<sub>2</sub>O<sub>2</sub> (LDH issuance) and the cellular death. In case of cellular apoptosis, the expression of phosphatidylserines negatively loaded at the surface of renal tubular cells, attract calcium positively loaded. There is ths initiated the formation of calcium crystals potentiating the effect of hyperoxaluria to produce renal lesions. Vitamin E significantly but only partially prevents the formation of ROS and the cellular lipidic peroxidation in tubular cells exposed to oxalates, but significantly inhibits the induction of the expression of the proteine TGF- $\beta$ 1, partially restores the catalase activity, GR, and the activity of enzyme GPx in the renal epithelial cells.

DFO seems to protect the cells against cytotoxicity at the iron and the raise of lipidic peroxidation. Vitamin E is a strong antiproliferative/antifibrogenic and antioxidant agent. The antioxidant therapy can prevent the adhesion and retention of the calcium oxalate in renal tubules through the protection against lipidic peroxidation induced by oxalates. Vitamin E decreases the production of TGF $\beta$ . Vitamin E, catalase and

desferoxamine reestablish the redox status perturbed by oxalates to a controllable level.<sup>18</sup>

### **Arterial hypertension of chronic renal failure (CRF)**

While the renal disease advances and there appears the renal functional alteration, the evolution to final stages of renal disease and raised tension values are accelerated. There is created a vicious circle where the aggravating factors are mutually influenced in the meaning of deterioration and there can be noticed, within all these pathological linkages newly created, a significant raise of oxidative stress. The total quantity of free radicals from the body increases through multiple mechanisms.

The oxidative stress is always present at the patients in the stage of predialise and at the patients in final stages of renal disease being in expectance of renal transplant.

The oxidative stress of CRF is characterized through an increased report GSSG/GSH, the presence of proteins with an advanced oxidation degree (AOPPs), with irreversible and nonfunctional structure accumulating in tissues and a raised level in the plasma of hydroperoxides and conjugated dienes; there is as well produced a down adjustment of the antioxidant system. In uremia there are accumulated AGEs and products with a high degree of lipoxidation. HTA of advanced stages of renal disease is dependent on the volume but there also appears RAS activation which also contributes to endothelial dysfunction and generation of free radicals.

NAD(P)H oxidase seems to be the most important source of ROS in the renal and cardiovascular system. In CRF there increase the subunits membranar gp91phox and p67phox cytoplasmatic of NAD(P)H-oxidase.

The majority of the patients with CRF have advanced atherosclerosis and especially coronary disease. The raise of peroxides production on NAD(P)H-oxidase are implied in formation pathogenesis and aterom plaque breakings. The ROS quantity in the body is increased in CRF and induces endothelial dysfunction and the stimulation of inflammatory cells. There takes place a process of LDL oxidation initiating the atherosclerosis process. The lipoproteins can be oxidized by free and linked metallic ions, ROS, peroxytrite, mieloperoxidase, lipooxygenase and disulfite of L-cysteine. Oxidized LDL-c improves the CD40 adhesion molecules expression on the lymphocytes T surface and of that of macrophages inducing and maintaining the inflammatory processes

at the level of the atherom plaque.<sup>21</sup> It would explain the raised incidence of ischemic cardiopathy in chronic renal failure.

Fixing Rac protein of GTP is activated by different proinflammatory substances and generates superoxide by NAD(P)H. Rac 1 is incriminate in the activation of NF-kB, cytokine forming and chemotactic substances, and the expression of adhesion molecules. VCAM-1 is important for atherosclerosis.

The carbonyl groups have a special role in atherosclerosis. HDL-c protects the body against the oxidative effect of LDL-c. HDL-c attenuates the expression of the adhesion molecules, induces the synthesis of NO and promotes vasorelaxation. Mieloperoxidase produces hypochlorous acid which oxidates HDL on way 3-chlorotyrosine and thus the cholesterol taking over from the periphery is affected.

As mentioned before the hemodialysis founding, these patients have a high degree of oxidation of LDL. The level of F2-isoprostanes is raised, but not because hemodialysis and promotes the synthesis of TGF- $\beta$ . The plasmatic albumine is also suffering from oxidation. On experimental animal models was showed as in IRC shows up an up-adjustment of NAD(P)H-oxidase and a down-adjustment of superoxide dismutase, catalase and glutathion peroxidase. The therapy with vitamin E and SOD-mimetic substances improves the oxidative stress and hypertension within this model. There are researches sustaining down adjustment of catalase and of glutathion peroxidase (key enzymes implied in the reduction and detoxification of hydrogen peroxide) at animals with CRF. This phenomenon can contribute to the accumulation of oxidized enzymes in CRF.

Frequently, patients with CRF present anemia requiring correction through i.v. administration of iron and erythropoetin.<sup>5,22</sup>

There are data showing that non-judicious administration of parenteral iron determines the raise of iron tissular deposits, renal and cardiovascular lesions. The iron excess at these patients is associated to the raise of cardiovascular risk and renal disease progression. These side effects are attributed to the catalyzer role in producing hydroxyl radicals (through Fenton reaction) and in lipid peroxidation at patients where antioxidant enzymes (superoxide dismutase, catalase and glutathion peroxidase) are in the red. Through this, iron produces lesions and cell death at renal and cardiovascular level and accelerates the atherosclerosis process. The raise of oxidative stress by iron administration promotes inflammation, endothelial dysfunction and NO deficit. Iron super loading accelerates the renal disease progression

determining glomerulosclerosis, tubular atrophy, interstitial fibrosis and iron deposits in glomeruls and in proximal and distal tubules.

There has been showed on experimental models that parenteral iron administration does not increase the activity of NAD(P)H-oxidase, contrary the administration of iron decreases the subunits p67phox and gp91phox at animals with CRF treated with iron. Consecutively, the lipid peroxidation intensification at iron administration does not seem to be due to superoxide generation by this enzyme, but to the catalytic effect of iron on lipid peroxidation and of the generation of hydroxyl radicals.

These data suggest that down adjustment of antioxidant enzymes can contribute to the aggravation of oxidative stress at iron loading at animals with CRF. It is interesting that despite a moderate increase in oxidative stress, a sole administration of iron i.v. at rats with CRF by reducing the renal mass does not significantly affect renal function in CRF if not added the systemic disease and comorbidities.

Anyway, raised deposits of iron or excessive administration of iron can lead to the excessive morbidity and mortality at hemodialized patients. Consequently, there is imposed precaution at the routine prescription of large doses of parenteral iron leading to supraloading.

The hemodialysis (HD) first corrects the volume dependency of HTA. In the moment of hemodialysis beginning, the complement activation due to dialyze membrane leads to release ROS of neutrophil and monocytes. The patients have high levels of reactive protein C, and raised production of chemotaxic substances. At the patients in HD program, the metalloproteinases level MMP-2 of the matrix and of TIMPs inhibitors have high levels especially at those with cardiovascular disease.

## DISCUSSION

Seeing the ROS role in hypertension of renal cause, there is the problem of therapies aimed with benefic effect regarding the tension values reduction as well as in remission or stopping of renal lesions.

There remains to estimate the role of antioxidant substances ( $\alpha$ -lipoic acid, ascorbic acid, vitamin E, SOD-mimetic substances) in the therapy of hypertension of inflammatory renal affections as glomerulonephritis or tubulointerstitial affections, where oxidative stress is high.

There are recent studies evaluating the role of anti-inflammatory substances as mycofenolate mofetil

which, by reducing renal interstitial inflammation depending on NF- $\kappa$ B activation by ROS, determine TA normalization at animals of experience and the reappearance of inflammatory infiltrate and increase of TA at SHR at stopping the treatment.<sup>2,20</sup>

The role of statines will have to be estimated in HTA treatment, and especially in that of the inflammatory renal disease, as on experimental studies on animals comparatively to , the antagonists of Ag II receptors and the inhibitors of aldosteron receptors, the statines have improved not only the biochemical parameters regarding the renal function and the estimation of extracellular matrix but also the histologic aspect of the lesions.<sup>12,23-25</sup>

Within this material, there is offered the theoretical support for the benefic role of angiotensin-converting inhibitors (ACEI) and angiotensin-II receptor blocker (ARB), regarding the control of blood pressure but also the improvement of the endothelial dysfunction, of proteinuria, renal lesions and not last in the reduction of oxidative stress. It seems that ACEI and ARB association does not supplementary reduce tension values but have an additive effect regarding the antioxidant effect implied in nephroprotection and cardioprotection.<sup>20,21,26</sup>

The judicious usage of parenteral iron in order to correct or prevent iron depletion, must constitute a central component of anemia management at the patients with final stages of renal disease.<sup>5,22</sup>

## **CONCLUSIONS**

The kidney has an important role in arterial hypertension being the cause and the aim of the disease as well. They know now that essential arterial hypertension induces sub-clinical renal lesion maintaining the increase of blood pressure. As well as in essential arterial hypertension and in renal hypertension, the oxidative stress is an always present link of the maintenance and evolution mechanism of the disease.

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