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New Insights into the Mechanism of Aminoglycoside Nephrotoxicity

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Abstract and Introduction

Abstract

Nephrotoxicity is one of the most important side effects and therapeutical limitations of aminoglycoside antibiotics, especially gentamicin. Despite rigorous patient monitoring, nephrotoxicity appears in 10–25% of therapeutic courses. Traditionally, aminoglycoside nephrotoxicity has been considered to result mainly from tubular damage. Both lethal and sub-lethal alterations in tubular cells handicap reabsorption and, in severe cases, may lead to a significant tubular obstruction. However, a reduced glomerular filtration is necessary to explain the symptoms of the disease. Reduced filtration is not solely the result of tubular obstruction and tubular malfunction, resulting in tubuloglomerular feedback activation; renal vasoconstriction and mesangial contraction are also crucial to fully explain aminoglycoside nephrotoxicity. This review critically presents an integrative view on the interactions of tubular, glomerular, and vascular effects of gentamicin, in the context of the most recent information available. Moreover, it discusses therapeutic perspectives for prevention of aminoglycoside nephrotoxicity derived from the pathophysiological knowledge.

Introduction: Aminoglycoside Antibiotics and Nephrotoxicity

Aminoglycoside antibiotics (AG) are widely used in the treatment of a variety of infections (for example, ocular, pulmonary, and intestinal infections) produced by Gram-negative bacteria and bacterial endocarditis.[1] Their cationic structure, which depends on the number of amino groups and on their distribution within the molecule, seems to have an important role in their toxicity, mostly affecting renal (nephrotoxicity[2]) and hearing (ototoxicity) tissues in which they accumulate. In spite of their undesirable toxic effects, AGs still constitute the only effective therapeutic alternative against germs insensitive to other antibiotics. This is primarily because of their chemical stability, fast bactericidal effect, synergy with betalactamic antibiotics, little resistance, and low cost.[3] In spite of being one of the most nephrotoxic AG, gentamicin is still frequently used as a first- and second-choice drug in a vast variety of clinical situations. Moreover, this aminoglycoside has been widely used as a model to study the nephrotoxicity of this family of drugs, both in experimental animals and human beings.[4–6] Most of the available data on the mechanisms responsible for AG nephrotoxicity has been obtained from gentamicin, especially at the preclinical level, in animal models or cell culture studies.

Although there are some reviews about the mechanisms explaining the toxic effects of gentamicin in the tubular epithelium, renal vasculature, and glomeruli, they lack an integrative view that brings together glomerular and tubular effects and their possible interplays. Thus, the purpose of this article is to review the effects of gentamicin in several kidney compartments with an integrative approach in order to further explain its nephrotoxicity.

Nephrotoxicity of Gentamicin

Incidence and Risk Factors

The incidence of aminoglycoside nephrotoxicity has progressively increased since its introduction, until reaching

10–25% of the treatments, despite the accurate control and follow-up exercised on patients.[5–9] Clinical studies lead to the conclusion that the incidence of renal damage varies depending on the target population,[10–13] which indicates that some individuals seem to be more sensitive than others. Table 1 shows the most important risk factors for the nephrotoxicity of gentamicin and, in general, of AGs.[14–17]

Table 1. Risk factors of aminoglycoside antibiotics related to patient and treatment characteristics, and to the concomitant administration of other drugs

Patient	Treatment	Other drugs
Older age	Longer treatment	NSAIDs
Reduced renal function	Higher dosage	Diuretics
Pregnancy	Split dosage	Amphotericin
Dehydration	—	Cisplatin
Renal mass reduction	—	Cyclosporin
Hypothyroidism	—	Iodide contrast media
Hepatic dysfunction	—	Vancomycin
Metabolic acidosis	—	Cephalosporin
Sodium depletion	—	—

Abbreviation: NSAIDs, nonsteroidal anti-inflammatory drugs.

Clinical Manifestations

The typical clinical manifestation of aminoglycoside toxicity is nonoliguric or even polyuric renal excretion dysfunction,[10,18–20] accompanied by an increase in plasma creatinine, urea and other metabolic products of the organism, proteinuria, enzymuria, aminoaciduria, glycosuria, and electrolyte alterations (hypercalciuria, hypermagnesuria, hypocalcemia, and hypomagnesemia).[21,22]

Tubular Effects

The tubular toxicity of gentamicin presents two aspects: (i) the death of tubular epithelial cells, mainly within the proximal segment, with a very important inflammatory component associated and (ii) the nonlethal, functional alteration of key cellular components involved in water and solute transport.

Mechanisms of Tubular Cell Death

A central aspect of aminoglycoside nephrotoxicity is their tubular cytotoxicity. Treatment of experimental animals with gentamicin results in apoptosis[23–25] as well as necrosis[26] of tubular epithelial cells. In culture, gentamicin also causes both apoptosis[27] and necrosis of these cells.[28] The phenotype of death might depend on the concentration of the drug, as with other cytotoxic compounds such as cisplatin and H_2O_2.[29,30] It might also depend on the concurrence of other triggering or predisposing factors, such as the degree of ischemia, on specific points of the renal parenchyma. Apoptosis is an ATP-requiring process. When the cell's ATP reserve drops, the death mode loses the typical characteristics of apoptosis and acquires those of necrosis.[31] Hypoxia inhibits respiration, ATP production, and sensitizes cells to Fas ligand[32] and induces cell death.[33,34] However, the most commonly observed phenotype *in vitro* is apoptosis, probably because it is necessary to expose cultured cells to high

concentrations of the drug (>1 to 2 mg/ml) to observe a modest cytotoxic effect.[28,35,36] Figure 1 graphically depicts the mechanisms of cytotoxicity detailed in the following paragraphs.

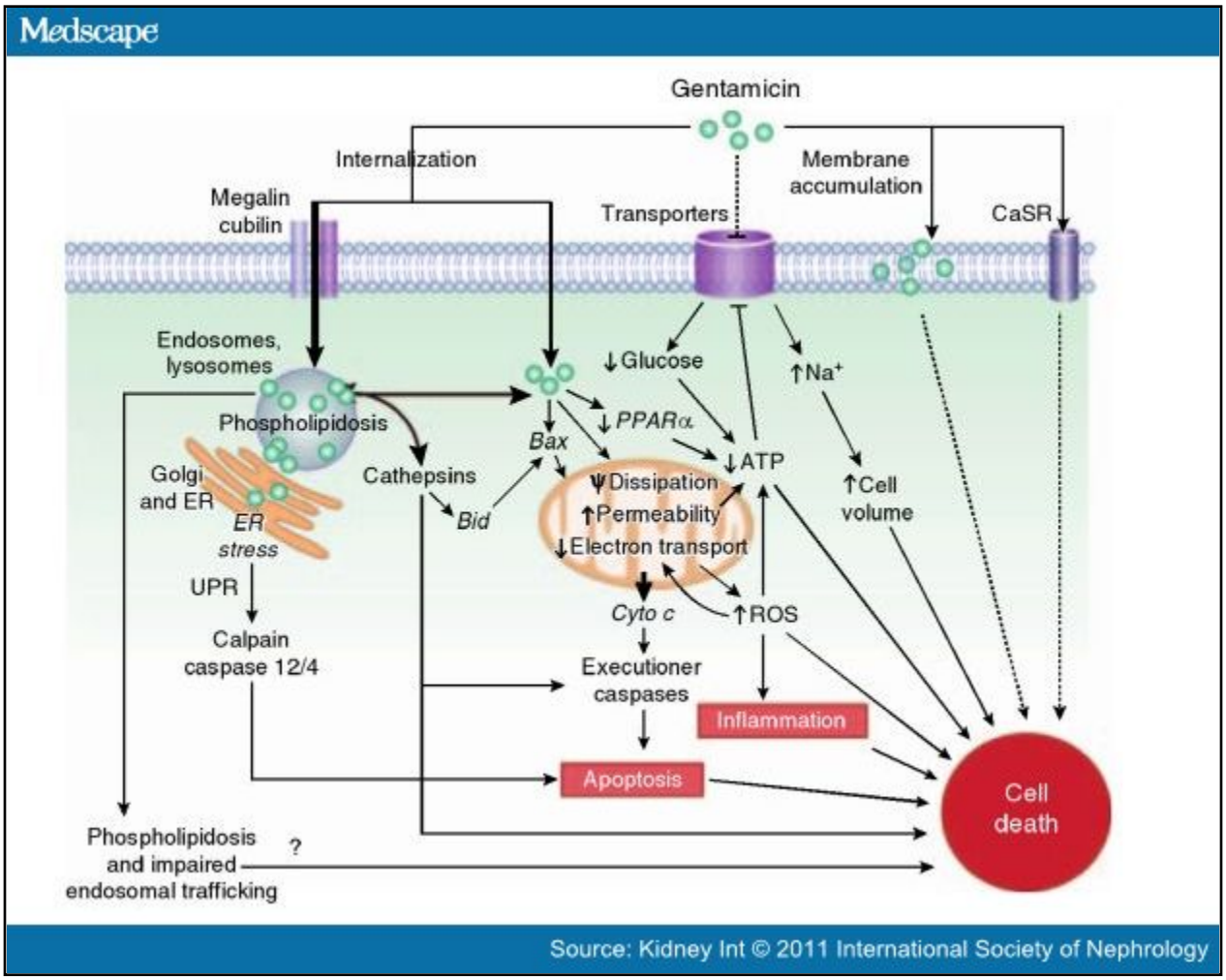

Figure 1. Mechanisms and cell signaling pathways underlying the cytotoxic effect of gentamicin. ATP, adenosine triphosphate; CaSR, extracellular calcium-sensing receptor; *Cyto c*, cytochrome *c*; ER, endoplasmic reticulum; *PPAR*α, peroxisome proliferator-activated receptor-α; ROS, reactive oxygen species; UPR, unfolded protein response; ?, The contribution of these mechanisms to cell death is not completely known.

Gentamicin cytotoxicity occurs in those cell types in which the drug accumulates. In the kidneys, these cells constitute the epithelial cells in the cortex, mainly in the proximal tubule of experimental animals[37] and humans,[16] and also in the distal and collecting ducts.[38] A higher accumulation of gentamicin in these cells is consistent with the expression of a transporter of proteins and cations, namely, the giant endocytic complex formed by megalin and cubilin, which is restricted to the proximal tubule. This complex is known to transport gentamicin and, in general, AGs, by endocytosis.[39] These drugs then traffic through the endosomal compartment and accumulate mostly in lysosomes, the Golgi, and endoplasmic reticulum.[40,41] Gentamicin binds to membrane phospholipids, alters their turnover and metabolism, and, as a consequence, causes a condition known as phospholipidosis that has been observed in humans[7] and experimental animals treated with the drug.[42,43] Lysosomal phospholipidosis results from (i) the reduction in the available negative charge necessary for the correct function of phospholipases[44] and (ii) inhibition of A1, A2, and C1 phospholipases.[4,45,46] Phospholipidosis correlates tightly with the level of toxicity of aminoglycosides.[43,47,48] Moreover, agents protecting from phospholipidosis, such as polyaspartic acid, also

prevent aminoglycoside nephrotoxicity.[49–51] However, the effect of polyaspartic acid has been ascribed to its capacity to bind gentamicin and thus to prevent its union to phospholipids.[52] Binding to phospholipids is also a requirement for gentamicin endocytosis,[53,54] indicating that further investigation is necessary to ascertain the exact role of phospholipidosis in tubular cell death.

When the concentration of aminoglycoside in endosomal structures exceeds an undetermined threshold, their membrane is disrupted and their content, along with the drug, is poured into the cytosol.[55,56] Cytosolic gentamicin then acts on mitochondria directly and indirectly,[57,58] and thus activates the intrinsic pathway of apoptosis, interrupts the respiratory chain, impairs ATP production,[58,59] and produces oxidative stress by increasing superoxide anions and hydroxyl radicals,[60,61] which further contributes to cell death. The indirect mitochondrial effect is mediated by increasing Bax levels[62] through the inhibition of its proteosomal degradation.[35] In addition, the lysosomal content bears highly active proteases named cathepsins, which are capable of producing cell death.[63] Cathepsin-mediated cell death occurs through apoptosis by directly cleaving active executioner caspases and indirectly unleashing the intrinsic pathway through the proteolytic activation of Bid.[64,65] In high amounts, cathepsins also cause a massive proteolysis that, especially under low ATP conditions, leads to a rapid, necrotic-like mode of cell death.[66]

In the endoplasmic reticulum, gentamicin inhibits protein synthesis,[67,68] impairs translational accuracy,[69] and might interfere with the correct posttranslational protein folding.[62] This generates endoplasmic reticulum stress and activates the unfolded protein response that, on continuous stimulation, activates apoptosis through calpains and caspase 12.[70–72] Finally, activation of the extracellular calcium-sensing receptor (CaSR) with gentamicin and other aminoglycosides has also been shown to induce a mild degree of apoptosis in CaSR-expressing tubule cells and not in those lacking it. However, CaSR is also expressed in gentamicin-resistant cells including bone, brain, colon, parathyroid gland, smooth muscle, endothelial cells, and so on. Clearly, more information is necessary to clarify the exact role and the relative weight of CaSR stimulation in tubule cell death induced by aminoglycosides.

Sub-lethal Alterations in Tubular Reabsorption

In experiments carried out with cultured cells or membrane vesicles from tubular cells, it has been shown that gentamicin, independently of cell injury, inhibits a variety of cell membrane transporters of both the brush-border and the basolateral membrane (reviewed in Mingeot-Leclercq and Tulkens[20]) including (i) Na-Pi cotransporter[73] and Na-H exchange;[74] (ii) carrier-mediated dipeptide transport;[75] (iii) electrogenic Na transport;[76] and (iv) Na-K adenosine triphosphatase.[77,78] Transport inhibition affects tubular reabsorption, but it may also compromise cell viability (Figure 1). For example, Na-K adenosine triphosphatase is a key component of cell volume homeostasis, and deregulated swelling may lead to necrosis or apoptosis.[79,80] As early as 30 min after gentamicin renal perfusion[21] or 3 h after gentamicin administration to rats,[81] deficient reuptake of calcium and magnesium is observed, leading to hypercalciuria, hypermagnesiuresis, and hypomagnesemia, before alterations in renal handling of Na^+ and K^+, and before detectable signs of renal damage and toxicity are evident. Gentamicin is transported by and also competes with proteins, organic cations, and other molecules for the megalin–cubilin endocytic complex in the proximal tubule, and thus impairs their reabsorption.[82–85]

Tubular Effects cannot Solely Explain the Reduced Glomerular Filtration Rate

The spilling of tissue and cellular residues to the tubular lumen partially or totally obstructs the tubules.[86,87] Tubular obstruction reduces, or even voids, the excretory function of the affected nephrons. In addition, it increases the hydrostatic pressure inside the tubule and in the Bowmans' capsule, which reduces filtration pressure gradient and, therefore, the glomerular filtration rate (GFR). Moreover, the increase of intratubular pressure increases the leak of the ultrafiltrate toward the interstitial space (backleak) and peritubular capillaries, and, thus, decreases excretion of the filtrate products.[86] Accordingly, tubular obstruction may account for a part of the reduced filtration caused by gentamicin. However, in mild cases and early stages of severe cases, that is, in the absence of

significant tubular obstruction, a relevant accumulation of creatinine and uremic products can be detected in the blood, which is usually the evidence that alerts on the underlying renal damage, and indicates that, by that time, GFR is already reduced. In the absence of significant nephron obstruction, an increase in plasma creatinine (and other products) can only be explained by a reduced GFR.

Tubular damage leads to a dysfunctional reabsorption process that produces an excessive delivery of water and electrolytes to the distal part of the nephron, which in turn triggers the tubuloglomerular feedback (TGF) mechanism. TGF is brought about by an angiotensin-II and adenosine-mediated afferent and efferent arteriole effects, and the subsequent decrease in GFR.[88,89] TGF is activated as a protective mechanism to avoid massive loss of water and electrolytes.[90] The TGF mechanism is known to adapt in a period of time ranging from 1 to 24 h.[91,92] Therefore, its role in the reduction of glomerular filtration should, theoretically, disappear after this interval. However, GFR continues to decrease as long as gentamicin treatment is maintained. As described in the following sections, oxidative stress, inflammation, and the release of vasoconstrictors induce mesangial and vascular contraction (see below). These may explain why GFR remains low even in the absence of an active TGF and of significant tubular obstruction. In addition, it can also be hypothesized that gentamicin might inhibit or modulate TGF-adaptive mechanisms.

Glomerular Effects

The glomerulus is the first part of the nephron to come into contact with chemical agents. Gentamicin has glomerular effects that alter filtration (Figure 2). (i) Gentamicin produces mesangial contraction (reviewed in Martínez-Salgado *et al.* [93]) and results in K_f (ultrafiltration coefficient) and GFR reduction;[94,95] (ii) gentamicin also stimulates mesangial proliferation paralleled by an increase in apoptosis of these cells, which basically compensate each other;[93,96] (iii) despite the fact that gentamicin does not generate significant morphological changes in the glomerulus, in high-dose treatments, a slight increase in size, alteration of their round shape and density, and a diffuse swelling of the filtration barrier associated with neutrophil infiltration have been detected,[97] although their pathophysiological significance is uncertain; and (iv) loss of glomerular filtration barrier selectivity, due to the neutralization of its negative charges,[98] contributes to proteinuria, especially under circumstances in which tubular reabsorption is impaired such as in tubular necrosis.

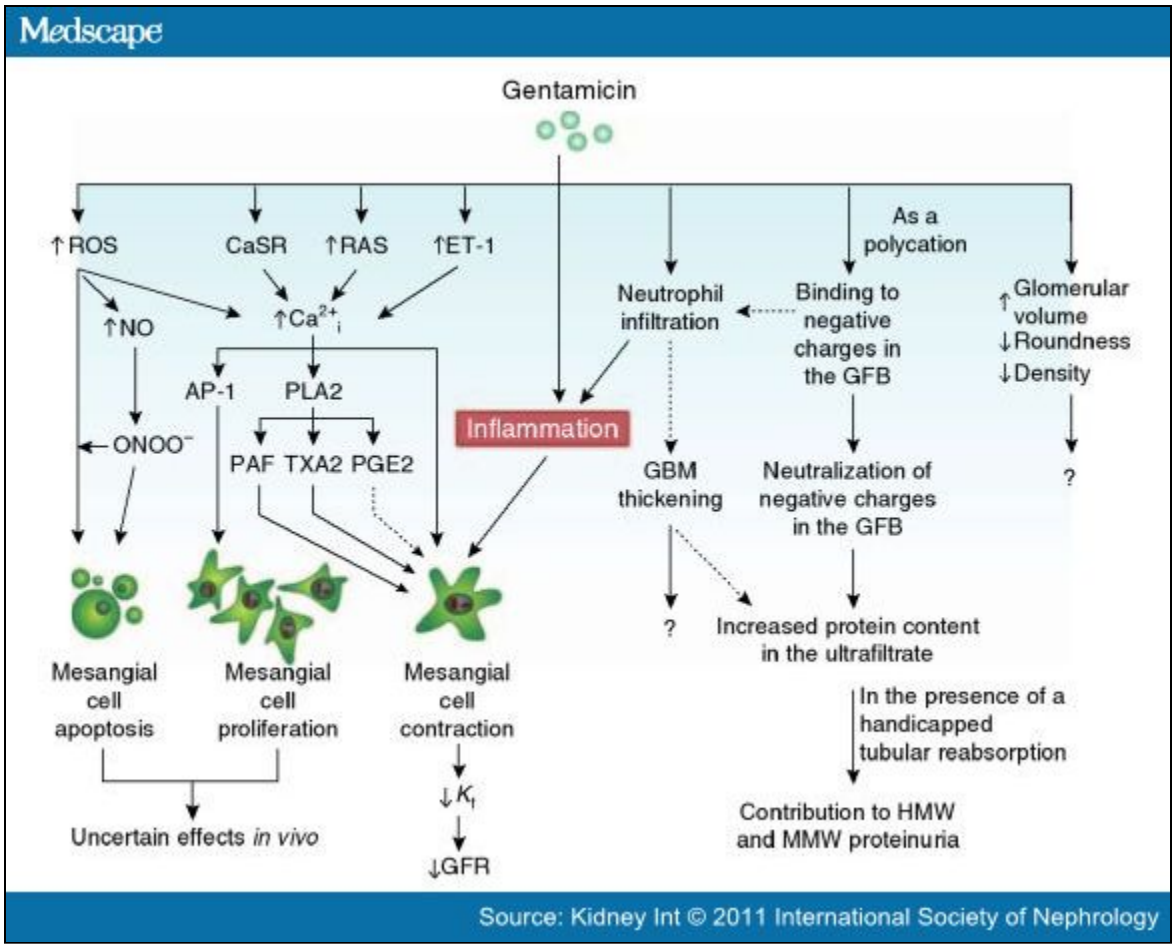

Figure 2. Glomerular effects of gentamicin. AP-1, activator protein 1; CaSR, extracellular calcium-sensing receptor; ET-1, endothelin-1; GBM, glomerular basement membrane; GFB, glomerular filtration barrier; GFR, glomerular filtration rate; HMW, high molecular weight; K_f, ultrafiltration coefficient; MMW, medium molecular weight; NO, nitric oxide; PAF, platelet activating factor; PGE2, prostaglandin E2; PLA2, phospholipase A2; RAS, renin–angiotensin system; ROS, reactive oxygen species; TXA2, thromboxane A2; ?, Unknown pathophysiological consequences.

Early studies demonstrated that gentamicin reduces the number and pore size of glomerular endothelial fenestrae,[99–101] correlating with a decrease in the sieving coefficient of low-molecular-weight proteins such as lysozyme,[100] and supporting a reduction in GFR. These effects seem to be the consequence of mesangial contraction. Gentamicin activates contraction of cultured mesangial cells and isolated glomeruli,[102,103] and thus reduces K_f. Several factors induced by gentamicin increase intracellular calcium concentration and cause mesangial cell contraction (reviewed in Martínez-Salgado *et al.*;[93] Figure 2). They include (i) platelet-activating factor (PAF) secretion and autocrine action;[102] (ii) activation of the renal renin–angiotensin system; (iii) production and action of vasoconstrictors such as endothelin-1 and thromboxane A2 arising from endothelial dysfunction or imbalance;[104] (iv) CaSR stimulation; and (v) increase in reactive oxygen species (ROS) production and oxidative stress.[105]

Activation of phospholipase A2 has also been associated with the synthesis of some of the above mediators and with the effect of gentamicin on mesangial cells.[103] Phospholipase A2 catalyzes the formation of arachidonic acid, a soluble phospholipid. Arachidonic acid generates, through cyclooxygenase, the synthesis of thromboxane A2 which leads to mesangial contraction. PAF is also synthesized from the soluble phospholipids that result from phospholipase A2 activity. PAF is recognized as an important mediator of mesangial contraction, which decreases *K*

$_f$ and GFR.[106–108] In fact, PAF antagonists partially inhibit gentamicin-induced reduction in GFR,[95,109,110] and mesangial contraction in isolated glomeruli and cultured mesangial cells.[92,102,110]

In rats treated with gentamicin, both proliferation and apoptosis take place at the same time in the mesangial compartment. Both effects apparently compensate one another, because no net variation in the number of mesangial cells has been reported.[93,111] Mesangial proliferation is mediated by calcium-dependent AP-1 activation.[96] Mesangial cell apoptosis is mediated by increased ROS[96,111] and probably by nitric oxide (NO) overproduction.[93] Gentamicin stimulates inducible nitric oxide synthase (iNOS) expression and NO production in isolated glomeruli and mesangial cells.[112–114] Excessive NO production due to expression of iNOS, especially under oxidative stress circumstances, interacts with superoxide anion to forms peroxynitrite, which causes nitrosative stress and cytotoxic effects.[115] The role of mutually counterbalancing mesangial apoptosis and proliferation is not clear. Probably, one is the homeostatic consequence of the other, in order to maintain tissue integrity. Gentamicin might cause a mild degree of apoptosis in mesangial cells followed by a repairing proliferation. Alternatively, gentamicin might promote the proliferation of mesangial cells (through the increment in Ca_i^{2+}) that, in the absence of tissue damage, would lead to apoptosis.[93] However, both increased proliferation and apoptosis have been detected in cultured mesangial cells treated with gentamicin,[111] which obscures both of these interpretations. As argued in Martínez-Salgado *et al.*,[93] *in vivo* the primary effect would be apoptosis, with subsequent homeostatic proliferation.

Vascular Effects

Gentamicin induces a reduction in renal blood flow (RBF),[116,117] which is the consequence of an increased resistance of the renal vascular bed rather than that of a lower perfusion pressure.[118] A lower RBF causes GFR to fall[119] (see Figure 3), and sensitizes tubule cells to cell death by reduction of oxygen and ATP availability (as explained above). RBF reduction arises initially (i) from the activation of TGF by the handicapped tubular reabsorption, in order to prevent massive fluid and electrolyte loss and (ii) progressively, superseding TGF adaptation, by production of vasoconstrictors within the renal vascular tree and mesangial compartment; and by direct effects of gentamicin on vascular cells (Figure 3).

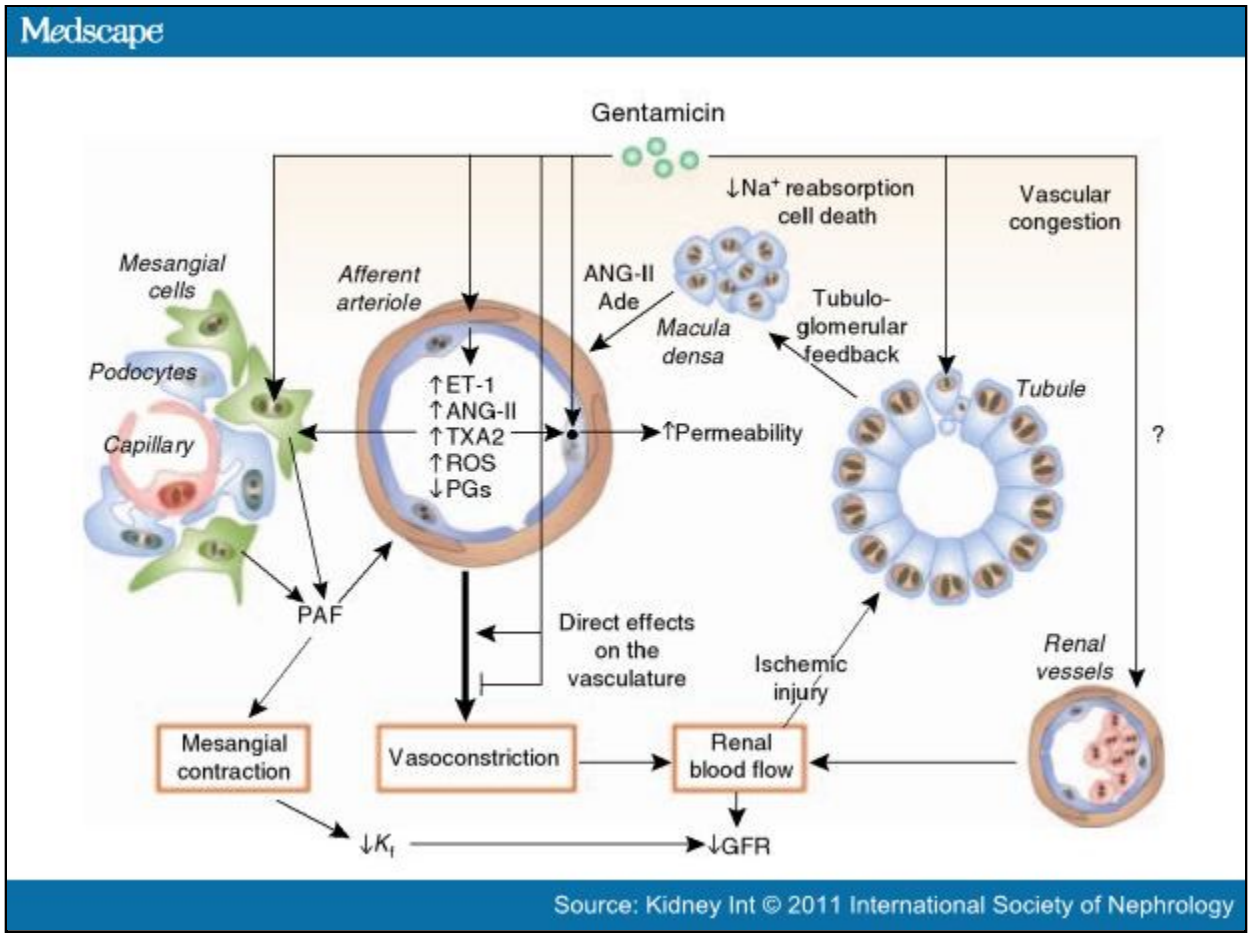

Figure 3. Vascular effects of gentamicin. Ade, adenosine; ANG-II, angiotensin-II; ET-1, endothelin-1; GFR, glomerular filtration rate; K_f, ultrafiltration coefficient; PAF, platelet-activating factor; PGs, prostaglandins; ROS, reactive oxygen species; TXA2, thromboxane A2.

The production of several vasocontrictors is increased on gentamicin treatment, including endothelin-1,[104] PAF, and arachidonic acid metabolites, mainly prostaglandins and thromboxane A2,[103,120,121] arising from endothelial and mesangial cells,[93] as explained in the previous section. They act in a paracrine manner on vascular myocytes and cause vasoconstriction. In addition to stimulating the production of vasoconstrictors, gentamicin also blocks the synthesis of vasodilator prostaglandins.[120] Endothelial NO synthase-derived NO, at low levels, mediates physiological vasodilatation, whereas excessive NO production due to the overexpression of iNOS (see above, section 'Vascular effects') can cause cytotoxic effects in surrounding cells. NO interacts with superoxide anion to form peroxynitrite, which induces protein and cell damage and uncouples endothelial NO synthase to become a dysfunctional superoxide-generating enzyme that contributes to vascular oxidative stress.[122]

Gentamicin also impairs vascular smooth muscle-relaxing capacity through an unraveled mechanism, theoretically contributing to vasoconstriction and RBF reduction, to an undetermined extent.[123] However, gentamicin has also been shown to relax isolated, precontracted arteries,[124,125] through the inhibition of phospholipase C, protein kinase C, and calcium movements.[124,125] This relaxing effect is exerted directly on smooth muscle cells and occurs despite gentamicin inhibiting the release of endothelium-derived relaxing factor, secondary to inhibition of PLC.[126]

Finally, leukocyte margination, leading to vascular plugging, congestion, and infarction, is induced by gentamicin in

retinal vessels after 48–72 h of treatment.[127] It can be speculated that vascular plugging contributing to ischemia might also occur in the kidneys, especially under a strong proinflammatory environment, although this has to be specifically corroborated.

Integrative Pathophysiology of Gentamicin Nephrotoxicity

Classically, the nephrotoxicity of gentamicin has been considered as a tubulopathy in which tubular damage and tubular dysfunction are the main cause of renal insufficiency. This may explain some clinical observations, such as proteinuria, enzymuria, and electrolytic alterations. However, as explained in the section 'Tubular effects cannot solely explain the reduced glomerular filtration rate', in the absence of tubular obstruction, tubular damage itself cannot account for a reduced GFR without the concourse of extratubular determinants. GFR reduction needs to be justified in order to fully explain the alterations in renal excretory function, leading to the accumulation of metabolic products in the blood, azotemia, uremia, and the whole renal syndrome produced by gentamicin.

Tubular and Glomerular Mechanisms differentially Contribute to the Reduced GFR

Tubular dysfunction leads to the loss of fluid and electrolytes that swiftly fire the TGF response, which reduces RBF and GFR to the appropriate level. Because, under physiological circumstances ~99% of water and electrolytes in the ultrafiltrate are reabsorbed along the tubule, a drastic reduction in GFR must be accomplished to compensate for a small reduction in tubular reabsorption, thus preventing the life-threatening loss of water and electrolytes. That is why even a mild injury to the tubular epithelium may bring about a pathological reduction in GFR and renal failure. However, TGF adapts within hours and its control over GFR is lost even in the presence of an increasing tubular incompetence. Yet, clinical and experimental observations demonstrate that, despite TGF adaptation, GFR grows lower as gentamicin-induced damage progresses, as described in previous sections.

Figure 4 shows the mechanisms leading to a reduced GFR. It can be observed that tubular malfunction leading to a defective reabsorption is the only mechanism that causes no GFR reduction directly, although it decreases GFR indirectly by activating the TGF mechanism, at least transitorily. Tubular obstruction increases progressively with tubular damage, as does its contribution to the reduced GFR. As such, it only partially explains the whole reduction in GFR, especially in the initial phase of acute kidney injury, which is the most relevant clinical situation. In these circumstances (Figure 5), a number of factors may hold GFR low in the absence of TGF-mediated control. Contracting factors produced by mesangial, vascular, and tubular cells, including ROS, PAF, angiotensin-II, and endothelin-1 act in an autocrine and paracrine manner to induce contraction of glomerular vessels and mesangial cells, which reduce RBF and K_f, respectively, and lower GFR. A question for the future is if a part of the reduction in GFR caused by gentamicin would still occur, should tubular alterations be completely and specifically prevented, or, whether most glomerular and vascular effects are, at least partially, independent of tubular damage. As explained above, gentamicin-induced mesangial activation and contraction have been documented in cultured, isolated mesangial cells,[93] indicating that no tubular-derived stimulation is necessary for these effects. In addition, reduced GFR and RBF may contribute to aggravating gentamicin-induced tubular damage,[128] probably because they limit oxygen and nutrient availability to tubular cells and facilitate oxidative stress, as it has been demonstrated in the ischemic renal failure.

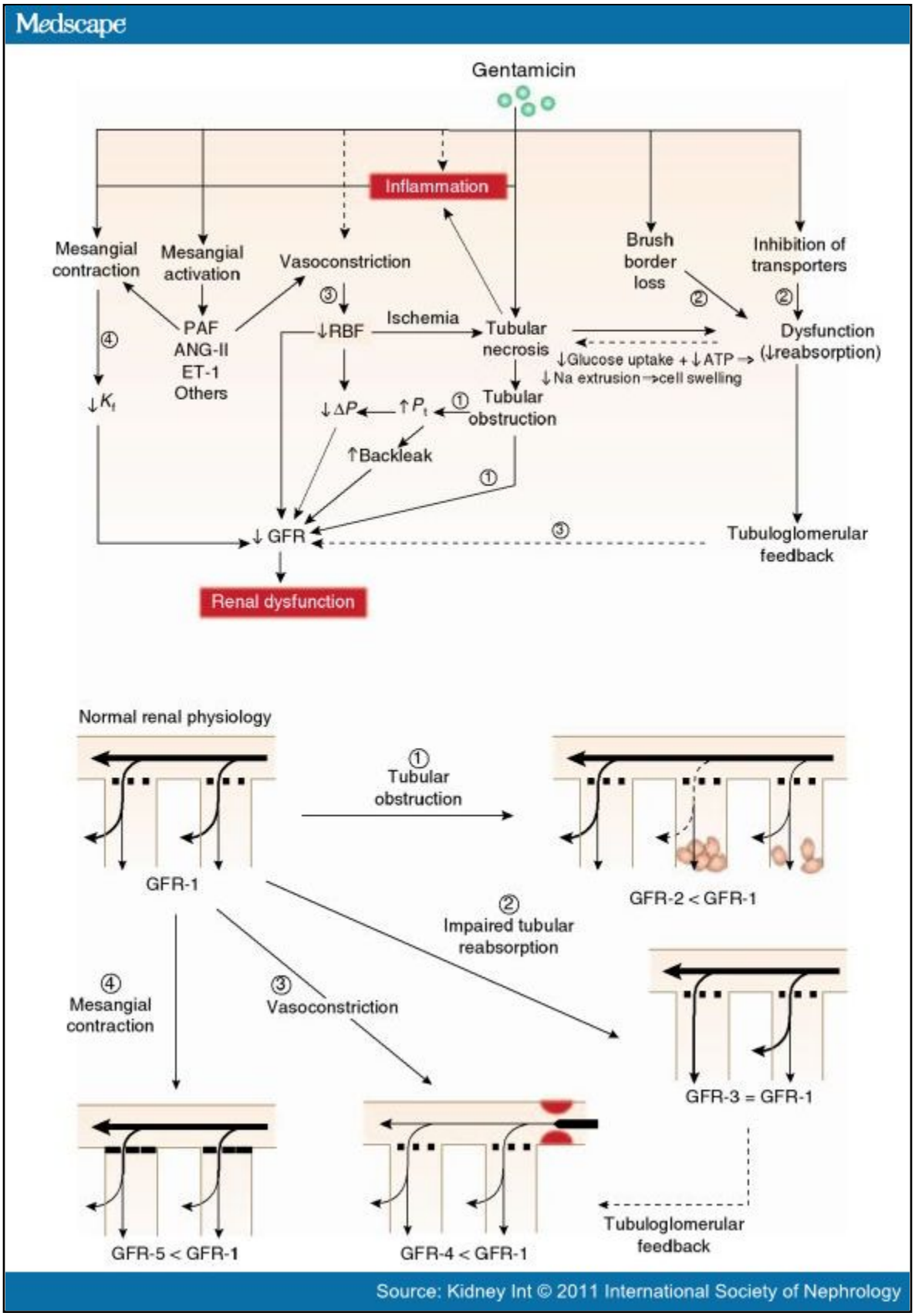

Figure 4. Integrative view of the mechanisms leading to gentamicin nephrotoxicity. It can be

appreciated that, in the absence of a significant tubular obstruction, vascular and mesangial mechanisms are necessary to explain the reduction in glomerular filtration (GFR) and renal excretion, once the tubuloglomerular feedback adapts. ANG-II, angiotensin-II; ATP, adenosine triphosphate; ET-1, endothelin-1; GFR, glomerular filtration rate; K_f, ultrafiltration coefficient; ΔP, net ultrafiltration pressure; PAF, platelet-activating factor; P_t, intratubular pressure; RBF, renal blood flow.

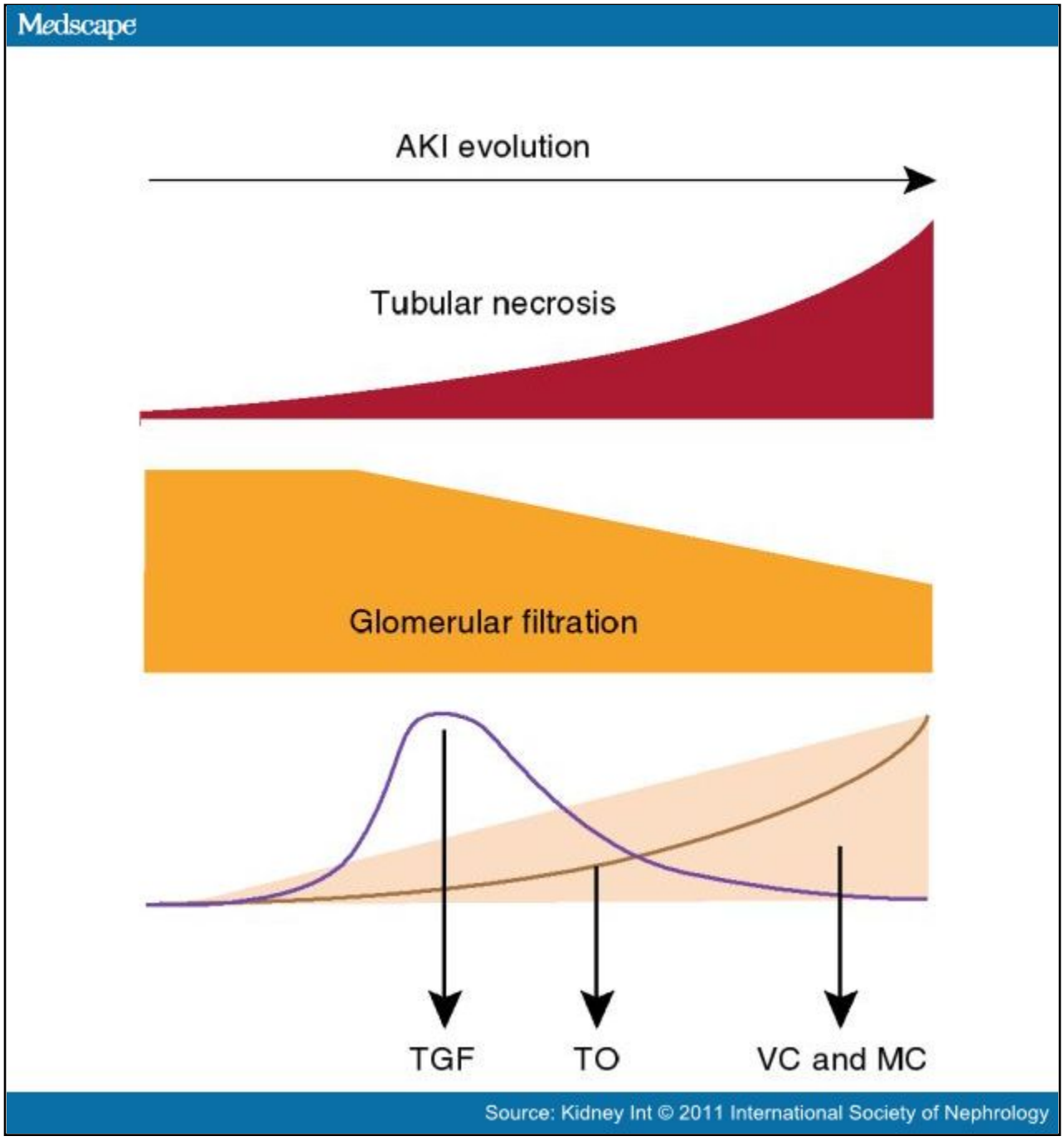

Figure 5. Comparative temporal evolution of the acute kidney injury (AKI), tubular necrosis, glomerular filtration, tubuloglomerular feedback, and vascular and mesangial contraction on treatment with gentamicin. Initially, tubuloglomerular feedback (TGF) controls glomerular filtration rate. As TGF adapts, increasing tubular obstruction (TO), and vascular and mesangial contraction (VC and MC) take over and make

GFR progressively lower.

Central Role of Oxidative Stress and Inflammation: A Loop of Damage Amplification and a Connection between Tubular and Glomerular Mechanisms

Oxidative stress has been suggested to have a key role in gentamicin nephrotoxicity.[129–131] This is mainly based on a myriad of studies conducted in experimental models demonstrating that cotreatment with a variety of antioxidants protects from gentamicin-induced renal damage,[61,117,132,133] although clinical data is not so conclusive.[134] Gentamicin directly increases the production of mitochondrial ROS,[58] which (i) are able of damaging many cellular molecules including proteins, lipids, and nucleic acids, thus impairing cell function and leading to cell death; (ii) contribute to mesangial and vascular contraction (as described in sections 'Glomerular effects' and 'Vascular effects'); and (iii) participate in inflammation.

The nephrotoxicity of gentamicin has been shown to involve an inflammatory response in experimental animals[135,136] and humans,[137] with cell infiltration, activation of resident cells, increased cytokine production,[138,139] and capillary hyperpermeability.[140] The inflammatory response, initially unleashed as a defense and repair mechanism, when globally considered seems to contribute to renal damage progression. In fact, strategies that protect from gentamicin-induced renal damage usually inhibit the inflammatory response.[135,141] In this sense, ROS are known to participate in the inception and signaling of inflammation,[142] which might explain why antioxidants are very effective at softening the renal damage inflicted by gentamicin[117,143,144] (Figure 6) and, in general, by other tubular necrosis-inducing nephrotoxins.[134,145] ROS such as superoxide anion[146] and hydrogen peroxide[147] activate nuclear factor κB, which has a key role in the inception of the inflammatory process. Indeed, nuclear factor κB inhibitors protect the kidney against gentamicin-induced damage.[148] Nuclear factor κB induces the expression of proinflammatory cytokines[149] and iNOS.[150] As described above, iNOS-derived NO can react with superoxide anion and produce peroxinitrite, a highly reactive radical that contributes to cell damage and reduced vascular relaxation.

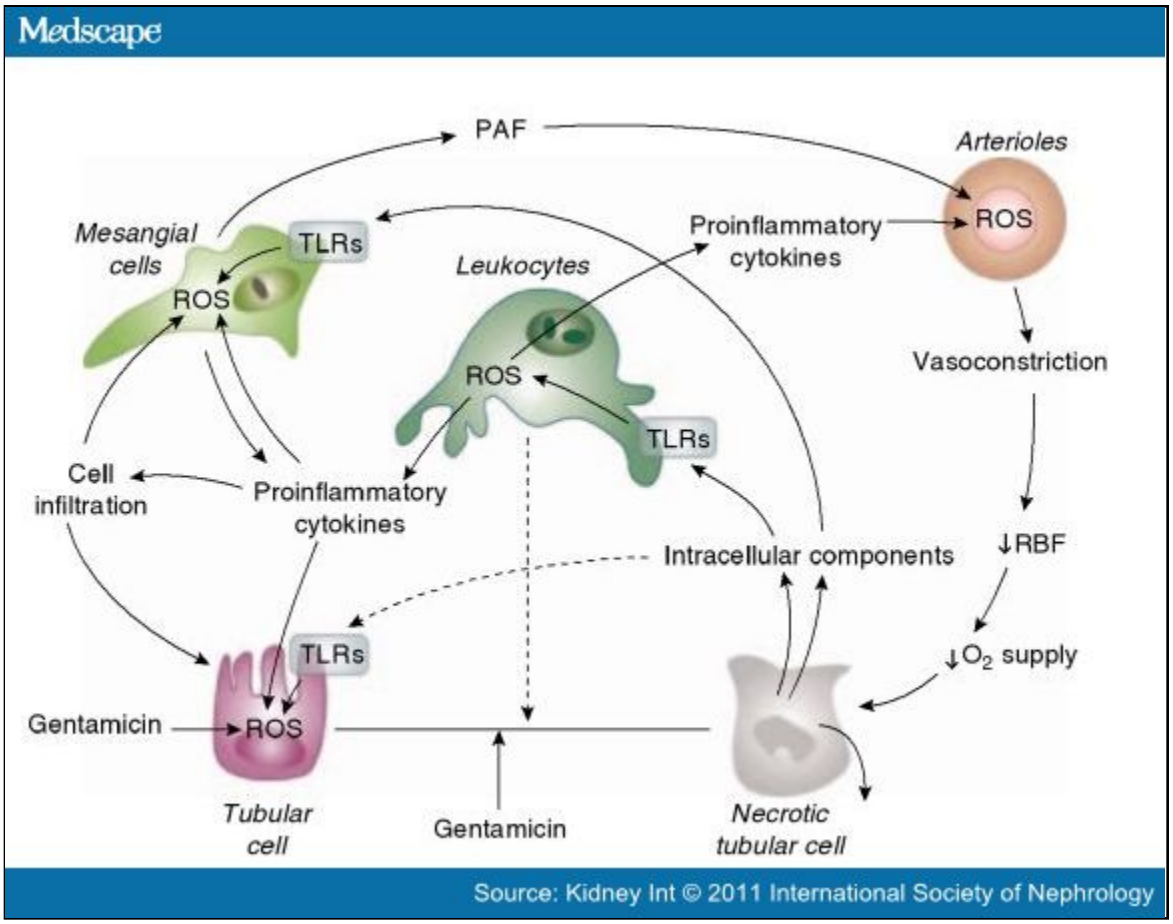

Figure 6. Role of inflammation in the amplification of tubular, glomerular, and vascular effects of gentamicin. PAF, platelet-activating factor; RBF, renal blood flow; ROS, reactive oxygen species; TLRs, toll-like receptors.

It can be speculated that the effect of antioxidants might be related to a combined action at different levels, including the following: (i) softening of gentamicin's direct cytotoxicity (as explained above); (ii) inhibiting vasoconstriction and mesangial contraction; and (iii) an antiinflammatory action. However, there is little information on the ability of antioxidants to modulate the direct cytotoxic effect of gentamicin on cultured tubule cells. To our knowledge, only Juan *et al.* [151] have reported a protective effect in this sense. In their article, tetramethylpyrazine reduces ROS accumulation and apoptotic events in rat renal NRK-52E cells. However, the effect of tetramethylpyrazine on cell viability is not reported. Because there are many apoptotic and necrotic pathways leading to cell death as a consequence of gentamicin action, and because their redundancy and hierarchical organization are not well understood, the magnitude of the direct cytoprotection afforded by ROS inhibition is unknown.

In any case, it is reasonable to think that the inflammatory response acts as an amplifying mechanism of damage (Figure 6). Initially, cell destruction through necrosis would lead to the onset of an inflammatory response. Tissue debris and cell content shed into the extracellular space trigger inflammation,[152] whereas an exaggerated inflammation would contribute to further damage that, in turn, would exacerbate the inflammatory response.[153] Inflammation also activates glomerular cells, such as mesangial cells, podocytes and epithelial cells, endothelial cells, and resident and infiltrated leukocytes. These, in turn, produce cytokines and growth factors that contribute to the pathophysiological process with different effects (Figure 6), including amplification of tubular damage.[154] As such, inflammation and oxidative stress provide a connection between tubular necrosis and glomerular and vascular activation and contraction, which ultimately further contribute to tubular damage, mainly through a reduction in RBF.

Clinical Implications for the Prevention of Nephrotoxicity

Prevention of nephrotoxicity is an unmet therapeutic objective that will improve the pharmacotoxicological profile and the clinical utility of many drugs significantly, including AGs. In many cases, nephrotoxicity is the most important limitation to the dosage or intensity of the therapeutic regimen, and may lead to serious health complications and even death in determined cases. Nephrotoxicity is a concern in all clinical settings, but takes special relevance among critically ill patients. Indeed, it is estimated that ~25% of the 100 most used drugs in intensive care units are potentially nephrotoxic,[155] and that nephrotoxicity is responsible for 10–20% of acute renal failure cases.[156] Besides a correct monitoring, maintenance of patient's hydration, and application of dialysis when necessary, there are no therapeutic tools available to prevent or palliate drug nephrotoxicity. There are no or very few tailored preventive strategies for individual nephrotoxic drugs, based on specific mechanisms of action. Nonetheless, this is another challenge for the future. In addition to the identification of less toxic compounds, several new strategies for the prevention of aminoglycoside nephrotoxicity are currently under different degrees of development, mostly at the preclinical level.

Inhibition of Tubular Accumulation

A proposed strategy focuses on finding drugs that prevent the accumulation of aminoglycosides by interfering with transport mechanisms. An obvious target is the megalin-related endocytic machinery responsible for AG transport and accumulation in tubular and auditory cells. Inhibition of aminoglycoside transport can be approached by administering (i) competitors for the receptor that displace aminoglycosides from binding to it or (ii) specific inhibitors of this endocytic pathway. Certain protein, fragments thereof and basic peptide ligands of megalin reduce the accumulation of gentamicin in cultured tubular cells and renal tubuli *in vivo* by inhibiting drug binding to the brush border.[85,157,158] Statins have been shown to reduce gentamicin accumulation in tubule cells and renal damage through a mechanism involving geranyl isoprenoids.[159] Megalin-mediated endocytosis involves other proteins with binding, adaptor, and unknown functions, such as cubilin, disabled-2, nonmuscle myosin heavy chain IIA and β-actin, which seem to participate in endocytic trafficking.[160] These proteins, and others resulting from a deeper knowledge of the endocytic mechanisms, are potential targets for pharmacological prevention of aminoglycoside accumulation. Indeed, genetic disruption of myosin VI[161] or treatment with the myosin inhibitor blebbistatin[160] reduces the uptake of proteins transported by the megalin complex. Myosin VI knockout mice show albuminuria with no alterations in urine output or electrolyte excretion. These initial results show a potential avenue for further exploration. Yet, the clinical consequences (for example, proteinuria) of interfering with megalin-mediated endocytosis as a mechanism of nephroprotection need to be determined in the short- and long term. In this line, myosin VI knockout mice show tubular dilation and fibrosis, consistent with persistent proteinuria.[161]

Cotreatment with Renoprotective Drugs

Another strategy relies on nephroprotective drugs for cotreatment along with aminoglycosides. At the preclinical level, many molecules have been shown to exert protective effects on drug nephrotoxicity and, specifically on aminoglycoside nephrotoxicity. By far, most of the studies have tested the ability of antioxidants to alleviate aminoglycoside nephrotoxicity. With one exception studied in patients,[162] all of them have been conducted in experimental animals. Preclinical studies offer unambiguous information on the beneficial effects of antioxidants. However, these results need to be further explored in the clinical setting, as promising, although inconsistent, results have been obtained on the protection exerted by antioxidants on the nephrotoxicity of other drugs.[134] In most studies in which inflammation has been evaluated, it is concluded that they might exert their effects through a cytoprotective and antiinflammatory action.

Improvement of RBF may also attenuate aminoglycoside nephrotoxicity, even independently from tubular damage.[162] An increased RBF by preglomerular or general vasodilatation can enhance GFR and attenuate the tubular damage caused or amplified by the reduced flow. In this sense, promising results have been obtained in

animals with PAF inhibitors, although they have not progressed further into human investigation. This could be an attractive strategy to pursue. In general, vasodilators also relax mesangial cells and augment K_f. As such, the increase in GFR is not only the result of hemodynamic improvement but also of K_f modulation. Thromboxane A2 inhibitors have been used in one study with protective results.[120] Calcium antagonists have also been used with contradictory results at the preclinical level. We have found only two studies conducted in humans. They document protection afforded by calcium channel blockers verapamil and nifedipine on gentamicin nephrotoxicity.[162,163] The effect of calcium antagonists may depend on the relative level of contraction of preglomerular and postglomerular vessels and mesangial cells, and on the weight of vasoconstriction and mesangial contraction in the overall effect of a determined experimental or clinical therapeutic regimen with aminoglycosides. This, in turn, may also depend on the dose and length of treatment, drug accumulation, and so on. A note of caution should also be introduced here, because the clinical consequences of augmenting GFR without a parallel amelioration of tubular damage may result in massive proteinuria, and water and electrolytic loss, which need to be addressed.

Other Strategies

Another potentially nephroprotective effect that should be pursued is the blockade of the immune response. In fact, genetic knockdown of toll-like receptor-4 has been shown to alleviate the renal lesion induced by cisplatin[164] and ischemia reperfusion[165] in mice, in which inflammation has a central pathological role. Indeed, ROS are involved in toll-like receptor-mediated inflammation.[166] Perhaps, cocktails containing several drugs aimed at providing protection against tubular damage and inflammation, and improvement of renal hemodynamics should be evaluated at the preclinical and clinical levels.

Conclusions and Perspectives

An integration of tubular, glomerular, and vascular effects of aminoglycosides based on the evidence discussed in this paper is consistent with an important component of tubular injury. In severe degrees of acute kidney injury induced by gentamicin, tubular obstruction may account, at least partly, for the reduced GFR. However, in mild cases and early stages of severe cases, that is, in the absence of significant tubular obstruction, GFR reduction can only be explained by extratubular mechanisms, namely, mesangial and vascular contraction. These later result from (i) the TGF mechanism, with the temporal restriction explained in section 'Tubular effects cannot solely explain the reduced glomerular filtration rate'; (ii) direct mesangial and vascular contraction; and (iii) indirect mesangial and vascular contraction produced by inflammation and paracrine mediators. Inflammation is known to result from tissue damage, specially arising from cell necrosis. Still, it remains to be elucidated (i) whether all the inflammatory responses are the consequences of tubular damage or whether they are also partly activated or amplified by tubular necrosis-independent mechanisms; and (ii) what is the contribution of direct extratubular effects of gentamicin to the overall syndrome, which are completely independent of tubular damage, and of mechanisms derived from tubular damage that alter glomerular and vascular function.

Finally, it should be stressed that known and new nephroprotective strategies should also be tested for their potential effects on the bactericidal effect of aminoglycosides. This issue has not been addressed in renal studies. For example, oxidative stress has been proposed to contribute to aminoglycoside bactericidal effect.[167] Then, treatment with antioxidants with the objective of reducing their nephrotoxicity may also impair their antibiotic activity. Thus, combined models of nephrotoxicity/nephroprotection and sepsis should be developed.

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