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Amelioration of aminoglycoside nephrotoxicity requires protection of renal mitochondria

Dmitry B. Zorov[1,2]

Cytotoxic effects of some therapeutic drugs, including those used for antibacterial treatment, can involve mitochondria by inducing their permeabilization, followed by cell death and organ malfunctioning. This suggests that a strategy to preserve tissue mitochondria could preserve normal organ functioning. Morales *et al.* reveal that the antidiabetic drug metformin prevents gentamicin-induced nephropathy through a mitochondria-dependent pathway, normalizing oxidative stress and restoring mitochondrial functional integrity.

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During the past two decades, a number of essential and unique functions of mitochondria have been discovered that are quite distinct from their commonly regarded functions as cellular 'power stations.'[1] Among these, the mitochondrial role in cell life/death decisions has received special attention.[2] The knowledge of such a critical role of mitochondria in cell death is of special significance because protecting mitochondria when it is needed has become a prosurvival cell strategy. While applicable to all kinds of cells, it is particularly relevant to post-mitotic cells with very low, if any, proliferative potential. In particular, the role of mitochondria in programmed cell death is associated with the release of apoptotic signaling molecules (such as cytochrome *c*, AIF, and SMAC/DIABLO). However, the production of reactive oxygen species (ROS) by mitochondria may also contribute significantly to any cell degradation process. The mechanisms of mitochondrial ROS production in cells remain incompletely understood, largely because the majority of studies revealing ROS production were made in model systems. This has hampered interpretation of experimental data with respect to its application to the behavior of mitochondria *in vivo* (or at least *in situ*). Whatever the mechanisms of mitochondrial ROS production, researchers have come to a general consensus that mitochondria represent one of the major cellular sources of ROS generation. When these ideas are applied to a higher cell organization, a great number of tissue pathologies, both inherited and acquired, were found to be associated with oxidative stress (for example, high ROS production); this emphasizes the critical role of mitochondria in these pathologies.[3] Among acquired tissue pathologies, chemically induced toxicity after pharmacological treatment deserves special consideration.

The use of aminoglycoside antibiotics (such as gentamicin) as therapeutic agents in the treatment of Gram-negative bacterial infections can be significantly limited by their nephrotoxicity[4] and ototoxicity. Apparently, ROS have been shown to play a key role in the toxicity of gentamicin resulting in acute kidney injury.

Morales *et al.*[5] (this issue) report that gentamicin-induced kidney injury is ameliorated by the widely used insulin-sensitizing biguanide metformin. Together with glibenclamide (an inhibitor of the adenosine triphosphate-dependent potassium channel), metformin is considered one of only two essential antidiabetic medicines by the World Health Organization. The antihyperglycemic properties of metformin are mainly attributed to the suppression of hepatic glucose production (through suppression of gluconeogenesis) and an increase in peripheral tissue insulin sensitivity. Although the exact mode of action of metformin has not been fully established, there is a set of data pointing to the direct or mediated mitochondrial effect of metformin. On one side, there is a claim that when it is used alone, the beneficial effect of metformin may be due to its mild inhibition of the mitochondrial respiratory chain (apparently of complex I).[6] Another set of data, including those described by Morales *et al.*,[5] demonstrates that metformin-based combination therapy can eliminate toxic side effects. This beneficial effect of metformin used in combination requires some alternative explanation.

A new look at gentamicin as a mitochondrial toxin that can exert its toxic effects when excreted by the kidney has apparently shed light on the complicated mechanisms of nephrotoxicity and nephroprotection, with mitochondria playing a critical role. Pharmacologically induced mitochondrial toxicity is often explained by a direct interference of the drug or agent with mitochondrial systems such as components of the electron transfer chain, oxidative phosphorylation, the transport system of oxidative substrates, or the machinery of mitochondrial DNA replication. Quite often mitochondrial toxicity can also be mediated by ROS. ROS are normally produced at low levels by mitochondria themselves, but under pathological conditions the intracellular and intramitochondrial ROS content may be amplified.[7] Under certain conditions, intracellular ROS content can reach a

[1]*Department of Bioenergetics, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia and* [2]*Laboratory of Cardiovascular Sciences, Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore, Maryland, USA*
Correspondence: *Dmitry B. Zorov, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119991, Russia.*
E-mail: zorov@genebee.msu.su or zorovd@mail.nih.gov

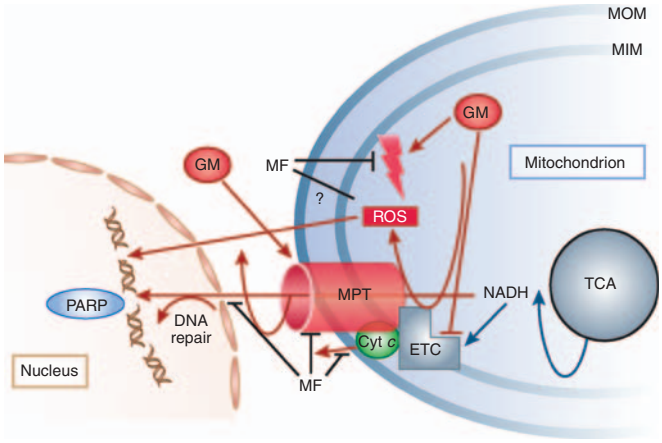

Figure 1 | Nephroprotective effect of metformin. Gentamicin (GM) attacks mitochondrial targets, causing induction of the mitochondrial permeability transition (MPT) and high production of reactive oxygen species (ROS). ROS attack numerous cellular targets, including DNA, leading to its breakage. MPT induction results in release of cytochrome *c* (Cyt *c*) and leakage and depletion of pyridine nucleotides (shown as NADH) due to their consumption in the DNA repair process by poly(ADP-ribose) polymerase (PARP). Mitochondrial depletion of cytochrome *c* and pyridine nucleotides causes mitochondrial respiratory inhibition due to inability of the electron transfer chain (ETC) to perform electron transport. Deleterious effects of gentamicin are shown by red arrows and objects. Metformin (MF) affords protection against gentamicin-induced injury apparently by affecting the primary target, the MPT pore, by preventing its opening. The possibility of intramitochondrial transport of metformin is shown by the question mark. MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane; TCA, tricarboxylic acid cycle.

toxic level (this was, in fact, observed after gentamicin treatment;[5] see Figure 1), which results in oxidative damage to elements essential for proper mitochondrial function, causing cell death and malfunctioning of the organ. The regulation of ROS homeostasis is complicated insofar as it involves maintaining a range below the threshold of damage but yet able to maintain certain key physiological functions of ROS as a redox signal required for proper activation of certain enzymes. This goal can be reached either by regulation of ROS production in mitochondria or by quenching of the excess ROS by redox buffers (when necessary, introduced into the system as exogenously supplemented antioxidants). Unfortunately, since the exact mechanisms of mitochondrial ROS production have not yet been defined, the regulation of ROS levels in the cell remains a difficult problem.

Besides ROS, another essential component that determines certain forms of cell death belongs to the mitochondrial structure or structures responsible for the so-called mitochondrial permeability transition (MPT), which occurs when the mitochondrial inner membrane, normally impermeable even for protons, becomes leaky for compounds up to approximately 1.5 kDa (for review, see Zorov *et al.*[8]). Morales *et al.*[5] have revealed a central role of the MPT in gentamicin-induced nephrotoxicity. Although a complete description of the specific mitochondrial structure or structures that can induce this phenomenon is lacking,[8] there are a great number of MPT inducers, including ROS, that have already been described. A few methods have become available to observe the MPT *in vitro* and *in situ*. Sensitivity to MPT inhibitors (cyclosporine A, methylAla(3)ethylVal(4)-cyclosporine (Debio 025), *N*-methyl-4-isoleucine cyclosporine (NIM811), Sanglifehrin A, bongkrekic acid, Ca^{2+} chelators, and so on) has become a rather powerful methodological approach to reporting the involvement of the MPT,[8] and this tool has been successfully used in the study by Morales *et al.*[5] (Figure 1).

A very important but frequently ignored result of MPT induction is that mitochondrial permeabilization causes the loss of essential mitochondrial ingredients necessary to perform a major mitochondrial function; that is, the process of electron transfer between respiratory complexes, required for proper coupling of oxidation with phosphorylation and formation of adenosine triphosphate, is lost. One important ingredient is cytochrome *c*, which is released from the mitochondrial intermembrane space, thus disrupting communication of mitochondrial electron transfer complexes III and IV. After its release from mitochondria, cytochrome *c* becomes an important proapoptotic factor.[2] Another factor that mitochondria lose as a result of MPT induction is a pool of pyridine nucleotides ($NAD(P)^+ + NAD(P)H$), essential cofactors of major mitochondrial dehydrogenases that provide the mitochondrial electron transfer chain with energy via electrochemical reducing equivalents. This pool is preferentially confined within mitochondria,[1,9] and its availability for donating protons to regulate oxidative phosphorylation is rate-limiting in the process of mitochondrial respiration. The depletion of this pool results in the ceasing of respiration, whereas the prevention of $NAD(P)^+$ loss by MPT inhibition helps support tissue viability.[1,10] Besides being an essential cofactor for cytosolic and mitochondrial dehydrogenases, pyridine nucleotides are the source of adenosine diphosphate (ADP)-ribose required for a very important signaling reaction, namely, ADP-ribosylation. Another NAD^+-consuming system is poly(ADP-ribose) polymerase, which is activated to repair DNA strand breaks accumulated after MPT-mediated apoptotic DNA degradation (Figure 1). These data point to an extremely important role of the MPT in drug cytotoxicity, which is exerted through multiple mechanisms apparently to ensure cell degradation.

The strength of the study by Morales *et al.*[5] is in a fresh look at old data highlighting the unwanted targeting of, and damage to, mitochondria by some pharmacological agents. It is quite remarkable that mitochondria, thought to ascend from ancient bacteria, are now found to be such a target of antibiotics used to treat Gram-negative bacterial infections. The authors have evidently caught what is usually missed in research exploring cytotoxicity. Fundamentally speaking,

they demonstrate that after gentamicin initiates the cell elimination process by targeting mitochondria, the result (cell death), after the 'point of no return' (mitochondrial permeabilization[2]) is passed, is realized through a number of different elements (enhanced ROS production, mitochondrial cytochrome *c* loss, NAD pool depletion) to guarantee the outcome. The mechanism by which metformin interferes with mitochondrial permeabilization in this case remains to be resolved; this understanding should facilitate the development of better ways to protect against unwanted cell death as a result of (unintended) mitochondrial injury.

DISCLOSURE
The author declared no competing interests.

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The riddle of the sphinx redux

James A. Shayman[1]

Understanding the mechanisms of glucocorticoid-mediated inhibition of inflammation has been challenging. This is particularly true with regard to the development of drugs that mimic the anti-inflammatory benefits of steroids while avoiding the untoward metabolic effects. Förster *et al.* report that the inhibition of stress-induced mesangial-cell apoptosis by dexamethasone is mediated by sphingosine-1-phosphate. These findings identify alternative pathways whereby the anti-inflammatory mechanisms of glucocorticoids can be probed.

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Glucocorticoids (GCs), introduced 60 years ago for the treatment of inflammatory and autoimmune disorders, remain a mainstay of therapy for various renal diseases. Indeed, the rate of mortality from systemic lupus erythematosus fell precipitously following the introduction of steroids in the early 1950s. Remarkably, these agents remain a primary therapy for lupus nephritis. Steroids are limited by the wide range of untoward effects, including Cushing's syndrome, dyslipidemia, hypertension, pancreatitis, immunosuppression, bone necrosis and osteoporosis, muscle atrophy, cataracts, and hypogonadism.[1] Consequently, the identification of GCs with greater selectivity and specificity has been a Holy Grail for many pharmacologists.

It is disappointing, then, that despite extensive efforts expended in pursuit of this goal, very limited progress has been made in the development of such agents. The absence of progress is understandable as greater insight has been gained in discerning the anti-inflammatory mechanisms of action of GCs. One of the greatest hurdles in the development of more selective anti-inflammatory agents lies in the GC receptor itself. The levels of the receptor protein are regulated in both tissue-specific and cell cycle-specific fashions. Additionally, several splice and translation variants of the protein have been identified.

There are three primary mechanisms of action of GCs:[2] direct cortisol–GC receptor DNA interactions; protein interference mechanisms secondary to the transcription of gene products that interact with the cortisol–GC receptor complex; and nongenomic pathways, that is, the interaction of GCs with membrane receptors and second messengers. Each of these mechanisms has been well studied in exploring the anti-inflammatory mechanisms of GCs. Examples of GC-mediated inflammatory mechanisms include the inhibition of prostaglandin production by the repression of cyclooxygenase-2; the induction of MAPK phosphatase I, leading to the dephosphorylation and inactivation of Jun N-terminal kinase; and the direct physical interaction of the cortisol–GC receptor complex with nuclear factor-κB. The last mechanism is particular important because nuclear factor-κB induces the transcription of cyclooxygenase-2, as well as several cytokines, chemokines, and cell adhesion molecules.

Therefore, there are many levels at which the anti-inflammatory effects of GCs can be understood, and many sites at which GC homologs could be designed to increase specificity and potency. Examples

[1]*Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA*
Correspondence: *James A. Shayman, Room 1560 MSRBII, 1150 West Medical Center Drive, Ann Arbor, Michigan 48109–0676, USA. E-mail: jshayman@umich.edu*