Toxicology 385 (2017) 10–17

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Key role of oxidative stress in animal models of aminoglycoside nephrotoxicity revealed by a systematic analysis of the antioxidant-to-nephroprotective correlation

Alfredo G. Casanova[a,b,c,d,1], Laura Vicente-Vicente[a,b,c,d,1], María Teresa Hernández-Sánchez[a,b,c,d], Moisés Pescador[a,c,d], Marta Prieto[a,b,c,d], Carlos Martínez-Salgado[a,b,c,d], Ana I. Morales[a,b,c,d], Francisco J. López-Hernández[a,b,c,d,*]

[a] Unidad de Toxicología, Departamento de Fisiología y Farmacología, University of Salamanca, 37007, Salamanca, Spain
[b] Institute of Biomedical Research of Salamanca (IBSAL-IESCYL), Salamanca, Spain
[c] Group of Translational Research on Renal and Cardiovascular Diseases (TRECARD), Salamanca, Spain
[d] Grupo de Investigación Biomédica en Cuidados Críticos (BioCritic), Valladolid, Spain

ARTICLE INFO

Keywords:
AKI
Aminoglycoside
Antioxidant
Correlation
Nephroprotection
Nephrotoxicity

ABSTRACT

The clinical utility of aminoglycoside antibiotics is partly limited by their nephrotoxicity. Co-administration of a variety of candidate nephroprotectants has been tested at the preclinical level. According to a recent meta-analytic study, antioxidants are the only family of compounds with enough preclinical documentation to draw solid conclusions on their class nephroprotective capacity in animal models. In this study a systematic analysis of the relation between the level of antioxidation and the level of nephroprotection was performed. A regression model is presented which crosses the y-axis (i.e. the axis representing the level of nephroprotection) very nearly the zero value, meaning that maximal prevention of the oxidative stress induced by aminoglycosides results in almost maximal nephroprotection. This indicates that oxidative stress plays a central role in the hierarchy of pathophysiological mechanisms underlying aminoglycoside nephrotoxicity. In addition, this model may potentially serve: i) as a standard to evaluate the role of the antioxidant effect of candidate nephroprotectants; ii) to reveal additional, antioxidant-independent effects among those compounds providing more nephroprotection than that expected from its antioxidant activity; and thus iii) to discriminate and focus most effective nephroprotectants on clinical usage.

1. Introduction

Aminoglycosides are wide spectrum antibiotics frequently used to treat infections caused by Gram-negative bacteria. However, their use is limited by their nephrotoxicity, which still appears in 10–25% of the therapeutic courses (Martínez-Salgado et al., 2007; Lopez-Novoa et al., 2011). These antibiotics accumulate in the renal cortex as they are endocytosed by the megalin-cubilin complex specifically present in proximal tubule epithelial cells, and further deposited in the endosomal compartment, alter the function of these cells and even lead them to death (Nagai et al., 2001). As a consequence, aminoglycoside nephrotoxicity results in acute tubular necrosis (Balakumar et al., 2010), with glomerular, renovascular and hemodynamic alterations, all of which lead to the reduction of glomerular filtration rate, azotemia and hydroelectrolytic imbalance (Lopez-Novoa et al., 2011). Prevention of aminoglycoside nephrotoxicity is an unmet clinical need. Posology adjustment provides some improvement, although undesired effects persist (Ali, 2003). Co-administration of potentially protective agents has identified a number of effective candidates at the preclinical level, but only a few clinical studies have been performed (Vicente-Vicente et al., 2017). More focused clinical research is necessary to identify the most effective nephroprotectants and their optimal administration route in humans, from the effective chemical portfolio tested in animal models.

Oxidative stress participates in the pathophysiological mechanisms underlying aminoglycoside nephrotoxicity (Ali, 1995; Tajiri et al., 1995; Abdel-Naim et al., 1999). Aminoglycosides stimulate mitochondrial production of reactive oxygen species (ROS; Morales et al., 2010), that, by oxidizing many cellular molecules including proteins, lipids, and nucleic acids (Cuzzocrea et al., 2002) cause (i) cell dysfunction and

* Corresponding author at: IBSAL – IECSCYL & USAL, Edificio Departamental, laboratorio S – 20, Campus Miguel de Unamuno, 37007 Salamanca, Spain.
E-mail address: flopezher@usal.es (F.J. López-Hernández).
[1] The authors wish to acknowledge that Alfredo G. Casanova and Dr. Laura Vicente-Vicente contributed as co-first authors to this paper.

http://dx.doi.org/10.1016/j.tox.2017.04.015
Received 4 March 2017; Received in revised form 27 April 2017; Accepted 30 April 2017
Available online 01 May 2017
0300-483X/

death; (ii) mesangial and vascular contraction; and (iii) inflammation (Cachofeiro et al., 2008). Aminoglycosides unleash a deleterious renal inflammatory response in experimental animals (Bledsoe et al., 2006; Kalayarasan et al., 2009) and humans (Kourilsky et al., 1982), with cell infiltration, activation of resident cells, increased cytokine production (Geleilete et al., 2002; Park et al., 2010), and capillary hyperpermeability (Goto et al., 2004). ROS such as superoxide anion (Schreck et al., 1991) and hydrogen peroxide (Meyer et al., 1993) activate nuclear factor kB, a key role mediator of the inflammatory process. Inducible nitric oxide synthase (iNOS)-derived nitric oxide (NO) reacts with superoxide anion to produce peroxynitrite, a highly reactive radical that contributes to cell damage and vascular contraction (Uppu et al., 2007).

Congruently with this, many antioxidants exert protective and preventive effects against aminoglycoside nephrotoxicity in animal models (reviewed in Lopez-Novoa et al., 2011). Reports involving antioxidants as effective nephroprotectants include a variety of distinct chemical entities, which suggests a class effect. In fact, a recent meta-analytic study (Vicente-Vicente et al., 2017) reveals that in animal models of aminoglycoside nephrotoxicity, antioxidants are indeed: 1) the most widely reported agents to produce a protective effect. 2) When considered as a whole, the most effective preventive strategy, over other families of drugs tested, including inhibitors of aminoglycoside accumulation in tubule cells (Schmitz et al., 2002), calcium channel blockers (Lee and Michael, 1985; Li et al., 2009) and vasodilators (Buyukafsar et al., 2001). And, 3) the only family of molecules from which solid conclusions can be drawn on their preclinical efficacy, based on the amount of data provided by the body of useful reports available.

In this study, we investigated in a systematic manner whether there exists a relation between the antioxidant and the nephroprotective effects of reported agents (i.e. those included in a previous meta-analytic study of nephroprotectants, Vicente-Vicente et al. (2017) on aminoglycoside nephrotoxicity, regardless of dosage, administration route, species, etc. A model linear relation was obtained, which will serve as a pattern to estimate whether a specific molecule or agent exerts more or less nephroprotective effect than that expected from its antioxidant capacity. It will also serve to elucidate the influence of oxidative stress in aminoglycoside nephrotoxicity. This approach excludes inter-experiment ambiguities, by relating directly the antioxidant effect achieved in each study by each candidate nephroprotectant, with the degree of nephroprotection obtained.

2. Material and methods

2.1. Retrieval of published preclinical studies

Reports of preclinical studies about protection against aminoglycoside nephrotoxicity published up to January 2016 were identified through an exhaustive search on Medline and Google Scholar. The combination of keywords used was “Aminoglycoside + prevention + nephrotoxicity‘; “Aminoglycoside + amelioration + nephrotoxicity’; ‘Aminoglycoside + prevention + kidney toxicity’ and “Aminoglycoside + amelioration + kidney toxicity”. The statistical analysis was performed only with the studies that met all the following characteristics: (1) To be preclinical studies using rat; mouse or rabbit species; (2) To provide drug; dose and route of administration; (3) To indicate the sample size of every compared group; and (4) To evaluate antioxidant nephroprotectants.

2.2. Generation of an antioxidation-to-nephroprotection correlation model

With the objective of evaluating a potential relation between the antioxidant and the nephroprotective activity of the nephroprotectants included in the study, the following parameters were defined:

- Nephroprotection ratio (E_{nep}):

$$E_{nep} = (\text{MaxNP-BasNP})/(\text{MaxNA-BasNA})$$

Where *MaxNP* is the value of the considered nephrotoxicity biomarker at the maximum toxicity time in the nephroprotectant + aminoglycoside group; *BasNP* is the value of the considered nephrotoxicity biomarker at the basal time in the nephroprotectant + aminoglycoside group; *MaxNA* is the value of the considered nephrotoxicity biomarker at the maximum toxicity time in the aminoglycoside group; and *BasNA* is the value of the considered nephrotoxicity biomarker at the basal time in the aminoglycoside group. Thereof, *MaxNP-BasNP* corresponds to the increment in the level of the nephrotoxicity biomarker considered, in the nephroprotectant + aminoglycoside group; and *MaxNA-BasNA* corresponds to the increment in the level of the same nephrotoxicity biomarker, in the aminoglycoside group. $E_{nep} < 1$ denotes nephroprotection (i.e. reduced aminoglycoside nephrotoxicity due to the action of the candidate nephroprotectant), being the lower the value of E_{nep} the higher the nephroprotective effect; $E_{nep} = 1$ means no effect exerted by the candidate nephroprotectant; whereas $E_{nep} > 1$ denotes increased nephrotoxicity caused by the candidate nephroprotectant.

- Oxidative stress ratio (E_{oxi}):

$$E_{oxi} = (\text{MaxOP-BasOP})/(\text{MaxOA-BasOA})$$

Where *MaxOP* is the value of the considered oxidative stress biomarker at the maximum toxicity time in the nephroprotectant + aminoglycoside group; *BasOP* is the value of the considered oxidative stress biomarker at the basal time in the nephroprotectant + aminoglycoside group; *MaxOA* is the value of the considered oxidative stress biomarker at the maximum toxicity time in the aminoglycoside group; and *BasOA* is the value of the considered oxidative stress biomarker at the basal time in the aminoglycoside group. Thereof, *MaxOP-BasOP* corresponds to the increment in the level of the oxidative stress biomarker considered, in the nephroprotectant + aminoglycoside-treated group; and *MaxOA-BasOA* corresponds to the increment in the level of the same oxidative stress biomarker, in the aminoglycoside-treated group. $E_{oxi} < 1$ denotes antioxidant activity due to the protector, being the lower the value of E_{oxi} the higher the antioxidant effect; $E_{oxi} = 1$ means no antioxidant effect exerted by the nephroprotectant candidate; and $E_{oxi} > 1$ denotes further oxidative stress caused by the nephroprotectant candidate.

In the present study, only the compounds that showed a significant antioxidant and nephroprotective effect in a previous meta-analysis (Vicente-Vicente et al., 2017) were included to build the first mathematical model. The E_{oxi} versus E_{nep} relation was represented for each biomarker of oxidative stress [i.e. malonyldialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT) and glutathione peroxidase (GPX)] versus each biomarker of renal damage [plasma creatinine and blood urea nitrogen (BUN)]. A preliminary correlation model for each of these pairs was obtained. Pearson correlation coefficient was calculated in each case. Only pairs of oxidative stress biomarker-renal damage parameter that contained values from 9 or more studies (i.e. published, included articles) and whose correlation p-value was lower than 0.05 were chosen. The correlation analysis was developed using the IBM SPSS™ (version 20) software.

For those models selected in the previous step, linear and non-linear regression equations were calculated. The best fit model was chosen and its 90% confidence area was calculated. This further allowed us to classify nephroprotectant candidates in and out of the limits posed by confidence intervals. The regression analysis was developed using software R™ version 3.0.3. Finally, a mathematical and graphical model