Experimental and Toxicologic Pathology 69 (2017) 83–92

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Carvedilol exacerbate gentamicin-induced kidney mitochondrial alterations in adult rat

Luís Félix[a,b,*], M.M. Oliveira[c], Romeu Videira[d], Elisabete Maciel[e,f], Nuno D. Alves[g], Fernando M. Nunes[c], Anabela Alves[h], José M. Almeida[i], M. Rosário M. Domingues[e], Francisco P. Peixoto[a,*]

[a] Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[b] Institute for Research and Innovation in Health (i3S), University of Porto (UP), Porto, Portugal
[c] Chemistry Centre of Vila Real (CQVR), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[d] REQUIMTE/Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto (UP), Porto, Portugal
[e] Mass Spectrometry Center, Department of Chemistry and QOPNA, University of Aveiro (UA), Aveiro, Portugal
[f] Department of Biology and CESAM, University of Aveiro (UA), Aveiro, Portugal
[g] Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho (UM), Braga, Portugal
[h] Animal and Veterinary Research Centre (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[i] School of Agrarian and Veterinary Sciences (ECAV), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

ARTICLE INFO

Article history:
Received 14 July 2016
Received in revised form 5 November 2016
Accepted 21 November 2016

Keywords:
Carvedilol
Gentamicin
Nephrotoxicity
Mitochondria
Carboxylic acid
Cardiolipin

ABSTRACT

Gentamicin is an aminoglycoside antibiotic widely used to treat many types of bacterial infections. Although its properties, his clinical use is limited due to the occurrence of nephrotoxicity, which has been related to mitochondrial dysfunction. Carvedilol, an antihypertensive drug with strong antioxidant properties, has been tested in order to prevent gentamicin nephrotoxicity. This study aimed to test this hypothesis using a rat model of gentamicin-induced nephrotoxicity. Animals were treated subcutaneously with DMSO (control) (0.4%/kg/24 h bw) for 11 days; with carvedilol (2 mg/kg/24 h bw) for 11 days; with gentamicin (60 mg/kg/24 h bw) for the last 8 days and with carvedilol (2 mg/kg/24 h bw) for 11 days and with gentamicin (60 mg/kg/24 h bw) for the last 8 days. Estimations of urine creatinine, urine carboxylic acids, blood urea, serum creatinine and glomerular filtration rate were carried out after the last administered dose of gentamicin. Mitochondria functionality was analyzed by monitoring its bioenergetics function and cardiolipin oxidized products were analyzed by ESI–MS. The kidneys were also examined for morphological changes. Gentamicin caused marked nephrotoxicity and mitochondrial dysfunction as evidenced by several mitochondrial parameters. Carvedilol did not induce significant changes while the co-treatment exacerbated the negative effect of gentamicin although maintaining ATP levels and membrane potential. Kidneys from gentamicin treated rats, with and without carvedilol, showed necrosis of tubular cells in renal cortex. Higher values on relative abundance of cardiolipin oxidation products identified as $[M\text{-}2H]^{2-}$ ions, at m/z 771 were observed in the groups treated with gentamicin. The observed effects were associated to a possible interaction of carvedilol with F_1F_0-ATP synthase that merit further investigation. In conclusion, carvedilol may contribute to the exacerbation of renal dysfunction induced by gentamicin, at least in some physiological and biochemical parameters. From a clinical perspective, and until further conclusions, cautious use of both drugs in combination is advised with particular emphasis in patients presenting mitochondrial disorders.

1. Introduction

Aminoglycosides, including gentamicin, are a widely prescribed and effective class of antibiotics used to treat serious bacterial infections, especially those caused by gram-negative and some gram-positive bacteria (Chen and Kaye, 2009). However, over the

* Corresponding authors at: Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal. Tel.: +351 259 350 475; fax: +351 259 350 629.
E-mail addresses: lfelix@utad.pt (L. Félix), fpeixoto@utad.pt (F.P. Peixoto).

http://dx.doi.org/10.1016/j.etp.2016.11.006
0940-2993/

years, numerous animal trials have reported side effects such as kidney injury, ototoxicity, and vestibular toxicity (Prezant et al., 1993). It is estimated that about 25% of the patients receiving therapeutic doses of aminoglycosides develop nephrotoxicity (Karahan et al., 2005; Lopez-Novoa et al., 2011). Besides being a complex phenomenon, its typical clinical manifestation is non-oliguric or even polyuric renal excretion dysfunction (Lopez-Novoa et al., 2011) characterized by an increase in serum creatinine, blood urea nitrogen concentration and severe proximal renal tubular necrosis, followed by deterioration and renal failure (Al-Majed et al., 2002; Cuzzocrea et al., 2002; Smetana et al., 1988). Moreover, both *in vitro* and *in vivo* studies showed the ability of gentamicin to interfere with mitochondrial functions (Walker and Shah, 1987; Yanagida et al., 2004) and suggest the role of free radical in gentamicin-induced nephrotoxicity as being the principal cause of toxicity by this compound (Erdem et al., 2000; Polat et al., 2006; Yaman and Balikci, 2009), thus, inducing apoptosis (Stojiljkovic et al., 2008). Although the side effects, aminoglycosides use has changed significantly in recent years as bacterial resistance has been increasing (Wargo and Edwards, 2014). Currently, aminoglycosides are often combined with other drugs (Ali, 2003) in an attempt to reduce its side effects which would have significant clinical value.

Carvedilol is a nonselective β-adrenergic receptor blocker that has been used to treat cardiac-related illnesses (Wang et al., 2014). Several properties have been described for this compound such as antioxidant, α_1 adrenoceptor blockade, vasodilatation, apoptosis inhibition (Savitz et al., 2000) and mitochondrial protection (Abreu et al., 2000). Carvedilol has also been shown to exert a neuroprotective effect in several models of transient focal stroke (Savitz et al., 2000), cardioprotective effect in experimental models of myocardial damage (Yuan et al., 2004) and nephroprotective effects (Padi and Chopra, 2002). These effects have been attributed partly to its antioxidant property, which emanates from the carbazole moiety that were reported to protect rats from gentamicin-induced nephrotoxicity (Kumar et al., 2000). However, the effects of carvedilol towards gentamicin-nephrotoxicity focusing on the structural and functional damage to the kidney mitochondria has not been explored.

Since mitochondrial damage may constitute an initial stage in antibiotic-induced nephrotoxicity (Wang et al., 2015), the present study was carried out to investigate the pharmacological potential of carvedilol against gentamicin-induced nephrotoxicity and the role that mitochondria plays in this process. This may be an interesting approach to understand or clarify the renal effects induced by the administration of these compounds. The results from this work could greatly contribute to the improvement in clinical quality and patients' treatment.

2. Materials and methods

2.1. Chemicals

An injectable solution of gentamicin sulfate in water (40 mg/mL) was obtained from Labesfal – Laboratórios de Almiro S.A. (Amadora, Portugal). Carvedilol was obtained from Roche Portugal (Amadora, Portugal), dissolved in dimethylsulfoxide (DMSO) and diluted to a concentration of 2 mg/mL in phosphate buffer saline. The DMSO concentration was kept to a minimum (Yuan et al., 2014). All other chemicals were of the highest grade commercially available and purchased from Sigma-Aldrich. Unless stated, solutions were prepared with ultra-pure water purified by a Milli-Q Gradient system (Millipore, Bedford, USA).

2.2. Experimental design

Male Wistar rats (weighing 200–300 g), obtained from Charles River (Barcelona, Spain), were used according with the National (DL 129/92; DL 197/96; P 1131/97) and European Convention for the Protection of Animals used for Experimental and Other Scientific Purposes and related European Legislation (OJ L 222, 24.8.1999) and approved by the Portuguese National Authority for Animal Health (General Directory of Veterinary Medicine). Animals were housed in a climate-controlled facility (temperature of 21 ± 2 °C, humidity of 60 ± 5%, a day/night cycle of 12 h, and *ad libitum* food and drinking water). Animals were accommodated to the laboratory conditions one week before being randomly divided in four groups (n = 6 each) as follows: (i) control group (CON): treated subcutaneously with DMSO (0.4%/kg/24 h bw) for 11 days; (ii) Carvedilol group (CAR): treated subcutaneously with carvedilol (2 mg/kg/24 h bw) for 11 days; (iii) Gentamicin group (GEN): treated subcutaneously with gentamicin (60 mg/kg/24 h bw) for the last 8 days; (iv) Carvedilol + Gentamicin group (CAR + GEN): treated subcutaneously with carvedilol (2 mg/kg/24 h bw) for 11 days and with gentamicin (60 mg/kg/24 h bw) for the last 8 days. A schematic diagram of this methodology is presented in Fig. 1. The body weight of animals was measured during the experimental period in order to observe symptoms of kidney disease the animals

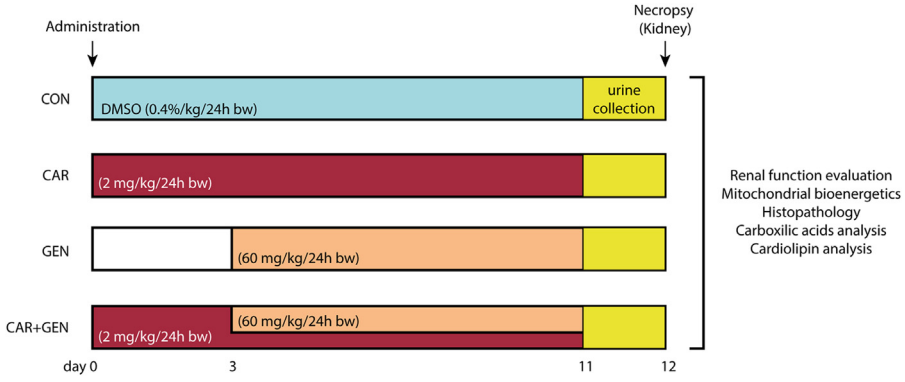

Fig. 1. Schematic diagram showing the timing of exposures and experimental analysis. Independent subcutaneous treatments with DMSO (0.4%/kg/24 h bw) for 11 days; with carvedilol (2 mg/kg/24 h bw) for 11 days; with gentamicin (60 mg/kg/24 h bw) for the last 8 days or with carvedilol (2 mg/kg/24 h bw) for 11 days and gentamicin (60 mg/kg/24 h bw) for the last 8 days were performed at 9 am. At the end of the treatments, the renal function as well as the carboxylic acids in urine samples were evaluated. Mitochondrial bioenergetics were also assessed. The influence of different treatments on kidney histopathology was studied. A thin layer chromatography experiment for analysis of lipids was also applied.