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Cardiotrophin-1 therapy prevents gentamicin-induced nephrotoxicity in rats

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ABSTRACT

Aminoglycosides are very effective antibiotics for the treatment of severe infections, but they rank among the most frequent causes of drug-induced nephrotoxicity. Thus, prevention of aminoglycoside nephrotoxicity is an unmet therapeutic objective. Cardiotrophin-1 (CT-1), a member of the IL-6 family of cytokines, has been reported to protect the kidney against toxic and ischemic acute kidney injury (AKI). We have assessed the effect of rat CT-1 in the severity of gentamicin (G)-induced AKI. Groups of male Wistar rats received the following for 6 consecutive days: i) isotonic saline solution (group CONT), ii) G, 150 mg/kg/day, i.p. (group G), iii) CT-1, 100 μg/kg/day i.v. (group CT-1), or iv) G and CT-1 at the doses described above. The G group showed a manifest AKI characterized by low creatinine clearance, high plasma creatinine and urea levels, increased urinary excretion of proteins, glucose and AKI markers such as *N*-acetyl-glucosaminidase, neutrophil gelatinase-associated lipocalin, kidney-injury molecule-1 and T-gelsolin, increased kidney levels of CD-68, iNOS, IL-1β and TNF-α, and markedly higher histological renal damage and leukocyte infiltration than the CONT and CT-1 groups. Administration of CT-1 together with G reduced almost all of the above-described manifestations of G-induced AKI. The results of this study have potential clinical application, as CT-1 is near to being used as a drug for organ protection.

1. Introduction

Drug nephrotoxicity is a very serious health and economic problem worldwide. It is the leading cause of intrinsic acute kidney injury (AKI) which represents 20% of all AKI cases. Aminoglycoside (AG) antibiotics are widely used and very effective against Gram-negative infections. The most important side effect of this family of drugs is nephrotoxicity [1–3]. Nephrotoxicity, ranging from a transient increase in plasma creatinine to severe AKI, occurs in approximately 10–25% of treated patients despite careful drug monitoring and the use of protective maneuvers [4–6]. AG-induced nephrotoxicity is caused by tubular lesions affecting mostly the proximal segment, which range from partial loss of the brush border to severe tubular necrosis [3,7,8]. The specificity of AG for renal toxicity is apparently related to its preferential accumulation in the renal proximal convoluted tubules [1,9]. Moreover, gentamicin (G) has been shown to exert functional glomerular [2,10–12] and vascular effects [13–15] that, depending on the dose, contribute to a larger or lesser extent to renal dysfunction [1,16].

In AG-induced AKI, renal inflammation is characterized by the infiltration of inflammatory cells, such as monocytes/macrophages, and the release of pro-inflammatory and chemoattractant cytokines. Deregulated renal inflammation is an important pathological process during AKI [1,9]. Activated proximal tubular epithelial cells also play a key role in releasing cytokines, chemokines, and cell adhesion molecules for the recruitment, retention, and activation of infiltrating cells at the inflammatory sites [9].

Prevention of nephrotoxicity is an unmet therapeutic objective that will significantly improve the pharmaco-toxicological profile

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and the clinical utility of many drugs, including AGs. In addition to the identification of less toxic compounds, new strategies for the prevention of AG nephrotoxicity relies on co-treatment with nephroprotective drugs. At a preclinical level, many molecules have been shown to exert protective effects on AG nephrotoxicity, but either they have not progressed to the clinical setting or they have failed to demonstrate a clear protective effect after clinical evaluation [17]. Envisaged renoprotection might encompass multi-inhibition of different key mechanisms of nephrotoxicity, whereas most compounds assayed so far have effects focused on a specific mechanism or signaling pathway. Cardiotrophin-1 (CT-1) is a cardio-active protein belonging to the interleukin-6 family, which also includes leukemia-inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), and IL-11. CT-1 signals through the LIF receptor (LIFR) complex consisting of the gp130/LIFRβ heterodimer [18]. CT-1 protects the heart from ischemic [19–21] and doxorubicin-induced damage [22]. CT-1 protects the liver from ischemia/reperfusion-induced damage [23], galactosamine-induced acute hepatic failure [24], acute liver failure after subtotal hepatectomy [25], and death associated with major surgical resection of the liver in cirrhotic rats [26]. CT-1 also has potent survival-promoting effects on neurons both in vitro and in vivo [27–30]. Additionally, CT-1 administration protects the kidney against AKI induced by ischemia/reperfusion [31] or iodinated contrast [32]. Thus, CT-1 seems to protect several organs against toxic or ischemic damage [33]. With this background, the purpose of this study was to assess the effect of rat CT-1 on the development of AKI caused by G.

2. Materials and methods

2.1. Reagents

Unless otherwise indicated, all reagents were purchased from Sigma (Madrid, Spain). Rat CT-1 (M.W. 21.5 kDa; batch 019R2500106543) was obtained from DRO Biosystems (San Sebastian, Spain) and stored at −80 °C until use.

2.2. Experimental design

Male Wistar rats (weighing 225–250 g; Harlan Laboratories, Barcelona, Spain) were used according to the European Guide for the Care and Use of Laboratory Animals (Directive 2003/65/CE) and Spanish national and regional regulations (Law 32/2007/Spain and RD 266/1998/CyL). Rats were divided into 4 experimental groups (n = 6 each): 1. Control group (CONT): rats receiving saline solution. 2. Gentamicin (G) group: rats receiving G (150 mg/kg/day, by intraperitoneal injection) for 6 days. This schedule was obtained in preliminary studies showing that this treatment produced a manifest AKI [34,35]. 3. Cardiotrophin (CT-1) group: rats receiving CT-1 i.v. (100 μg/kg/day) for 6 days. 4. Gentamicin + Cardiotrophin (G + CT-1) group: rats receiving G for 6 days and CT-1 for 6 days from the day before G administration (same doses as in the previous groups). The doses of CT-1 were determined in preliminary studies as those demonstrating renoprotective effects without significant effects on arterial pressure [31,32]. To test renal function, urine and blood samples were collected from a set of animals placed in metabolic cages, as previously described [36]. Blood (0.150 mL) was obtained every two days from a cut in the tail tip into heparinized capillaries. Plasma was obtained by centrifugation and stored at −80 °C. At the end of the experiments (day 7), blood samples were collected by aortic puncture, and the rats were perfused with cold isotonic saline solution through the abdominal aorta. Both kidneys were removed and halved, and a piece of tissue including cortex and medulla was trimmed down, fixed in 4% buffered paraformaldehyde for 24 h and embedded in paraffin for histological studies. Another portion of kidney was frozen in liquid nitrogen and conserved at −80 °C for other analytical determinations.

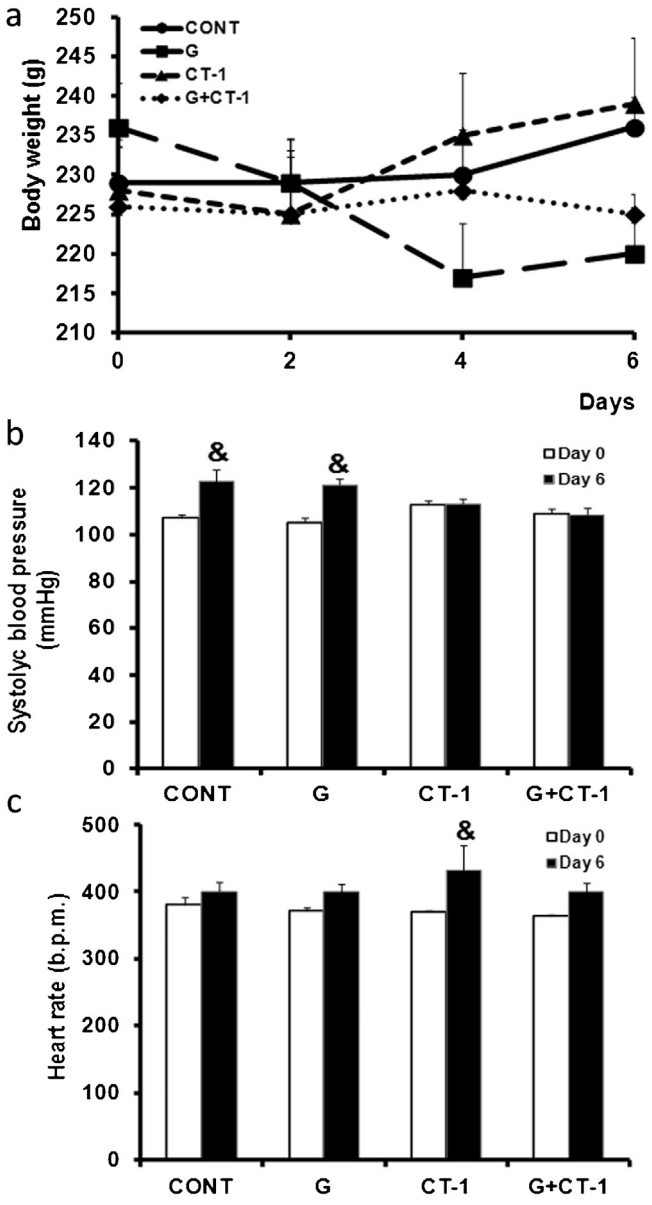

Fig. 1. Body weight (a), arterial pressure (b) and heart rate (c) in control rats (CONT) and rats treated with gentamicin (G), cardiotrophin-1 (CT-1) or both (G + CT-1). Symbols: &: statistically significant difference ($p < 0,05$) versus day 0.

2.3. Measurement of systolic blood pressure and heart rate

Systolic blood pressure and heart rate were measured in the tail by plethysmography using a NIPREM 546 blood pressure meter (Cibertec, Madrid, Spain) and recorded using a PowerLab 800 data acquisition system (AD Instruments; Dunedin, New Zealand) as previously described [37].

2.4. Biochemical analysis of renal function and renal damage markers

Urinary and plasma concentrations of urea and creatinine and urinary glucose concentration were measured using an automated method (Reflotron®, Roche), according to the manufacturer's