Gentamicin Nephrotoxicity – A Comparison of In Vitro Findings with In Vivo Experiments in Equines

M.R. van der Harst*, S. Bull, C.M. Laffont and W.R. Klein
Faculty of Veterinary Medicine, Department of Equine Sciences, Utrecht University,
Yalelaan 12, 3584 CM Utrecht, The Netherlands
*Correspondence: E-mail: m.r.vanderHarst@vet.uu.nl


ABSTRACT

The aminoglycoside gentamicin is often used in equine practice. Despite its clinical use, concerns remain regarding the potential toxic side-effects, such as nephrotoxicity, in equine patients, particularly after repeated dosing. The aim of the study was to investigate first in vitro the mechanisms contributing to the renal toxicity of gentamicin and to identify sensitive biomarkers indicating proximal tubule damage. To this end, the kidney-derived cell lines LLC-PK1 and MDCK were treated with gentamicin at different concentrations. Toxicity was assessed by measuring the release of gamma-glutamyl transferase (GGT), and the production of reactive oxygen species (ROS). Cell viability was measured using Alamar blue (AB) and Neutral red (NR) cytotoxicity assays. Gentamicin exerted a dose-dependent toxicity. Primarily, loss of brush border membrane integrity, indicated by GGT leakage, and an increased ROS production were observed. As GGT was found to be a sensitive marker for gentamicin-induced renal cell injury, in the subsequent in vivo experiments, in which ponies were given gentamicin (3.0 mg/kg bw three times daily and 4.5 mg/kg bw twice daily) for five consecutive days, plasma levels and the urinary excretion of GGT and creatinine were measured and the GGT/creatinine ratio was calculated. Elevated GGT levels in urine following gentamicin therapy were observed, but this enzyme leakage was transient and returned to baseline values after cessation of therapy. It could thus be concluded that even a conservative dose regimen of gentamicin did not result in significant renal toxicity in healthy ponies.

Keywords: dose regime, γ-glutamyl transferase, gentamicin, horses, nephrotoxicity

Abbreviations: AB, Almar blue; ANOVA, analysis of variance; ATCC, American Type Culture Collection; AUC, area under the curve; Cl, plasma clearance; Cmax, maximum plasma concentration; DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; GGT, gamma-glutamyl transferase; H2DCF, dichlorodihydrofluorescein diacetate; MDCK, Madin Darby canine kidney; MRT, mean residence time; NR, Neutral red; PBS, phosphate-buffered saline; ROS, reactive oxygen species; t1/2, half-life of distribution; t1/2e, half-life of elimination; Vc, volume of the central compartment; Vss, steady-state volume of distribution

INTRODUCTION

Gentamicin is a widely used aminoglycoside antibiotic, applied in equine medicine because of its bactericidal effects against Gram-negative and staphylococcal bacteria. Despite its frequent use, the potential nephrotoxicity of gentamicin has been a matter of concern in clinical pharmacotherapy. Renal toxicity of gentamicin is linked to its
accumulation in proximal tubule cells and is characterized by a mild rise in plasma creatinine levels, accompanied by an impairment of renal function in cases where prolonged therapy is needed. The relative toxicity of gentamicin appeared to correlate with the concentration in the renal cortex in experimental animals. Accumulation in renal tubule cells occurs following uptake via the organic anion transport system. As this is a saturable process, the degree of accumulation and toxicity is predominantly time-dependent and, to a lesser extent, depends on the individual dose given. This was exemplified in experiments demonstrating that a continuous infusion of gentamicin was more nephrotoxic than the same total dose given at intermittent time intervals (Powell et al., 1983).

Gentamicin-induced renal toxicity has been described in many experimental animal species, as well as in man (Frame et al., 1977; Riviere et al., 1983a,b; Houghton et al., 1986; Hinchcliff et al., 1989; Mattie et al., 1989; Gilbert, 1991; Prins et al., 1993). However, toxicity data in equines are scarce, as the majority of studies have described only the kinetics of gentamicin (Haddad et al., 1985; Hinchcliff et al., 1989; Clarke et al., 1992; Godber et al., 1995; Magdesian et al., 1998; Martin-Jimenez et al., 1998).

Hence, the aims of the present study were twofold. The first was to study the mechanisms of gentamicin toxicity in cells derived from either the proximal (LLC-PK1) or distal (MDCK) tubulus. Endpoints to describe gentamicin toxicity included the production of reactive oxygen species (ROS) (Trayner et al., 1995) and gamma-glutamyl transferase (GGT) release, as well as Alamar blue (AB) reduction and Neutral red (NR) uptake (Bull et al., 2001). Second, the toxicity and pharmacokinetics of gentamicin were evaluated in the dose regime applied in clinical practice for postsurgery equine patients and early sins of nephrotoxicity were monitored by measuring GGT:creatinine ratio in urine during the course of gentamicin treatment and by postmortem histopathological examinations.

MATERIALS AND METHODS

In vitro studies

Phosphate-buffered saline (PBS), M199 and DMEM/F12 media, l-glutamine and fetal calf serum (FCS), were purchased from Invitrogen (Breda, The Netherlands). Neutral red, l-glutamyl-p-nitroanilide, gentamicin free base and gentamicin sulphate were obtained from Sigma (St Louis, MO, USA). Alamar blue (AB) was purchased from Biosource (Etten Leur, The Netherlands). Dichlorodihydrofluorescein diacetate (H2DCF-DA-D390) probe was purchased from Molecular Probes (Leiden, The Netherlands). All other chemicals used were of analytical grade.

Cell lines and culture conditions

Porcine kidney LLC-PK1 (ATCC number CL-101) cells, derived from the proximal tubule, and canine kidney MDCK (CCL-34) cells, isolated from the distal convoluted