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Telbivudine attenuates gentamicin-induced kidney injury in rats

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ABSTRACT

Nephrotoxicity has been associated with nucleos(t)ide analogues other than telbivudine (LdT). This study investigated the potential effects of LdT and lamivudine (LAM) on renal function in an experimental rat model of gentamicin-induced acute nephrotoxicity. A total of 28 healthy Wistar albino rats were randomly divided into four experimental groups: negative control; positive control (PC); LdT; and LAM. Nephrotoxicity was induced by gentamicin in the LdT, LAM and PC groups. LdT and LAM were administered to two groups for 6 weeks starting on the ninth day. Blood samples were collected weekly and cystatin C levels were measured by ELISA. Animals were sacrificed on the 50th day and the kidneys were removed for histological examination. Serum cystatin C levels differed significantly between the LdT and LAM groups ($P < 0.007$) and between the LdT and PC groups ($P < 0.001$). Renal function was significantly improved in the LdT group at the start of antiviral treatment on Day 8 and at the end of treatment on Day 50 ($P = 0.001$ and 0.007). Glomerular injury, acute tubular necrosis and total injury score were significantly reduced in the LdT group relative to the PC and LAM groups upon histopathological examination. LdT was associated with significant improvements in renal function as measured by biochemical and histopathological methods. The acute kidney injury model data should be supported by clinical studies to suggest that LdT treatment may have advantages for patients with underlying chronic kidney disease receiving chronic hepatitis B treatment.

1. Introduction

Nucleos(t)ide analogues (NAs) have been licensed for oral treatment of chronic hepatitis B (CHB) [1] and are the most widely used antiviral drugs in CHB treatment in recent years. NAs inhibit viral DNA polymerase [2]. Although these agents are effective in the suppression of hepatitis B virus (HBV) replication, they cannot fully eliminate the virus from the host. As a result, long-term treatment is required in most patients. NAs are generally safe and well tolerated, although there are concerns of metabolic side effects in some patients with long-term use [3].

Treatment of CHB with NAs is long. Besides renal involvement during the course of hepatitis as an extrahepatic manifestation, long-term use of NAs may be associated with renal failure especially in patients with underlying renal disease, elderly patients and in those using concomitant nephrotoxic drugs. Adefovir (ADV), which nowadays is rarely used, carries a higher risk of nephrotoxicity. Tenofovir (TDF) may induce nephrotoxicity in nearly 15% of patients treated for 2–9 years [2].

NAs are excreted by the kidneys and as a result dosage adjustment according to glomerular filtration rate (GFR) is required [4]. Cohort studies have suggested that renal impairment is more frequently reported in CHB patients treated with ADV. In a longitudinal observational study of 214 patients receiving HBV treatment, the estimated GFR (eGFR) decreased in patients receiving ADV as monotherapy or in a combination but remained stable in patients receiving lamivudine (LAM), TDF or entecavir (ETV) at a median follow-up of 2.4 years. The eGFR decreased in patients with a baseline eGFR of <90 mL/min/1.73 m^2 regardless of treatment [5]. Of 641 patients initially randomised in a pivotal study of TDF, 585 entered the open-label phase, of which 10 (1.7%) had an elevation of serum creatinine of ≥0.5 mg/dL above baseline [6]. In another 3-year prospective study of 400 patients receiving TDF treatment, few patients (1.3%) experienced renal adverse reactions, and creatinine clearance

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(CL_{Cr}) remained stable over time. Patients responded favourably when the TDF dose was adjusted for decreased CL_{Cr} [7].

In a limited number of clinical studies conducted by different teams worldwide, telbivudine (LdT) has been reported to promote a significant increase in GFR [4,8–10]. These data suggest that LdT may protect against nephrotoxicity resulting from other conditions. A real-world retrospective study of 587 patients with CHB treated with TDF ($n = 170$), LdT ($n = 184$) or ETV ($n = 233$) showed that eGFR decreased significantly in the TDF group after a mean treatment duration of 17 months, however it increased in the LdT group after a mean treatment duration of 32 months. In the ETV group there was no significant change in eGFR after a mean of 44 months of treatment [11].

In a recent study including 4178 CHB patients treated with NAs, renal functional declined in 706 (16.9%). Age, hypertension, diabetes, history of liver or kidney transplantation, underlying chronic kidney disease and simultaneous administration of diuretics were found to increase the hazard ratio for renal functional decline. The eGFR significantly increased over time in patients receiving LdT or clevudine compared with LAM [12].

Cystatin C is a low-molecular-weight non-glycosylated protein and a cysteine proteinase inhibitor. It is synthesised continuously at a constant rate in all nucleated cells in the body [13]. Cystatin C is freely filtered through the glomeruli, re-absorbed through tubular epithelial cells and metabolised rapidly in the kidneys [14]. It is used for calculating GFR. More recently, cystatin C has been applied to monitor changes in GFR and has been shown to be more sensitive than traditional measures based on serum creatinine level and CL_{Cr} [15]. Gentamicin, an aminoglycoside antibiotic, is frequently used to induce experimental nephrotoxicity [16]. Cystatin C is reported to be the most sensitive test of nephrotoxicity in rats with nephropathy induced by gentamicin and can detect damage earlier than traditional creatinine and blood urea nitrogen assays [17]. Thus, cystatin C was preferred in the current study.

The potential effects of LdT and LAM on renal function were investigated in a model of experimental nephrotoxicity caused by gentamicin exposure. Serum cystatin C levels were measured as an indicator of nephrotoxicity, and histopathological changes in kidney tissue were examined.

2. Materials and methods

2.1. Animals

A total of 31 healthy Wistar albino male rats (age 12–16 weeks, weight 260–360 g) were used in the study. Standard chow and water were provided ad libitum during the study period. Animals were housed under a 12-h light/dark cycle.

2.2. Drugs

Gentamicin sulphate was administered by the intraperitoneal (i.p.) route for 8 days at a dose of 80 mg/kg to induce nephropathy [15]. The experimental drugs LdT (Sigma, St Louis, MO) [18] and LAM (Sigma) [19] were dissolved in distilled water and were administered orally by gastric gavage.

2.3. Experimental protocol

As a comparator to LdT, LAM was preferred since LAM has sufficient renal safety data, is inexpensive and is easily accessible in many developing and Far East countries. Three rats were used in a preliminary study to verify the nephrotoxicity model [pilot study (PS)]. Rats in the PS group were given 80 mg/kg gentamicin sulphate by i.p. injection once daily for 8 days. Necropsies were performed and nephrotoxicity was determined histopathologically on Day 9.

The principal investigation was initiated after confirming nephrotoxicity in the PS group. The study included four experimental groups as follows: negative control (NC) (i.p. saline only); positive control (PC) (i.p. gentamicin only); LdT (10 mg/kg); and LAM (5 mg/kg).

A total of seven rats were assigned to each group at random. Nephrotoxicity was induced by administering 80 mg/kg gentamicin sulphate to the LdT, LAM and PC groups by i.p. injection for 8 days [16]. Blood samples were collected from the tail vein in all groups at the end of Day 8. A dose of 10 mg/kg LdT was administered to the LdT group [17] and 5 mg/kg LAM was given to the LAM group [19] by gastric gavage for 6 weeks starting on Day 9. Blood was obtained from the tail vein of rats in the LdT, LAM and PC groups on Days 15, 22, 29, 36, 43 and 50. Blood was collected from the NC group on Day 50. The study protocol is presented in Fig. 1. Rats in all groups were weighed prior to blood collection and LdT and LAM dosing was adjusted according to body weight.

2.4. Serum cystatin C measurement

Blood samples were collected into microtubes and were centrifuged at $1000 \times g$ for 10 min at room temperature to separate serum for measurement of cystatin C. Serum samples were stored at –80 °C for subsequent analysis. Rat serum cystatin C levels were measured quantitatively using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (BioVendor–Laboratorni Medicina a.s., Brno, Czech Republic) according to the manufacturer's recommendations. Just prior to the assay, serum samples were diluted 500 times with dilution buffer in two steps. The performance of the ELISA kit was validated using low- and high-concentration control materials included by the manufacturer.

2.5. Histopathological examination of kidney tissues

PS group rats were sacrificed on Day 8 and rats from the remaining four groups were sacrificed on Day 50. The kidneys from rats in all four groups were excised following necropsy. A semiquantitative evaluation of renal tissues was performed by scoring the degree of damage severity according to previously published criteria [20]. The following parameters were used for the grading of injury: (i) glomerular injury (% of renal parenchyma involvement): none = 0, <25% of glomeruli exhibiting non-specific features of injury = +1, 25–50% of glomeruli exhibiting non-specific features of injury = +2, 50–75% of glomeruli exhibiting non-specific features of injury = +3, and >75% of glomeruli exhibiting non-specific features of injury = +4; (ii) acute tubular necrosis (% of renal parenchyma involvement): none = 0, <25% of tubules out of the entire renal parenchyma = +1, 25–50% of tubules out of the entire renal parenchyma = +2, 50–75% of tubules out of the entire renal parenchyma = +3, and >75% of tubules out of the entire renal parenchyma = +4; and (iii) tubulointerstitial inflammatory infiltrates: none = 0, leukocytes confined within the interstitium = +1, and leukocytes infiltrating the interstitium and tubular epithelial cells = +2 [20].

2.6. Ethical considerations

This study was approved by the Animal Experiments Local Ethics Committee of Ondokuz Mayıs University (Samsun, Turkey). Animal experiments were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All animal studies were performed in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.