A two-week regimen of high-dose integrase inhibitors does not cause nephrotoxicity in mice

Michael T Eadon^{1,2}, Hongji Zhang¹, Todd C Skaar²,
Takashi Hato¹, Pierre C Dagher¹, Samir K Gupta³
and Zeruesenay Desta²

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Abstract

Background: The integrase inhibitors, raltegravir and dolutegravir, are nucleoside reverse transcriptase inhibitor-sparing agents which may be used as part of first-line antiretroviral therapy for HIV. These drugs inhibit creatinine secretion through organic cation transporters, thus elevating serum creatinine without affecting glomerular filtration. We sought to determine whether subtle signs of nephrotoxicity could be observed in mice administered a two-week regimen of high-dose integrase inhibitors.

Methods: C57BL/6 mice were fed standard water (CTRL, $n = 6$), raltegravir-containing water (40 mg/kg/day, $n = 6$), or dolutegravir-containing water (2.7 mg/kg/day, $n = 6$) for two weeks and sacrificed. Endpoints were assessed including urine microalbumin, kidney injury molecule-1 renal tissue gene expression, renal histopathology, serum creatinine, and blood urea nitrogen.

Results: The results are NOT consistent with a direct nephrotoxic effect of the integrase inhibitors in mice. Serum creatinine was significantly elevated in raltegravir and dolutegravir mice ($p < 0.05$) compared to control (raltegravir = 0.25 mg/dl, dolutegravir = 0.30 mg/dl versus CTRL = 0.17 mg/dl). Blood urea nitrogen, cystatin C, and urine microalbumin were unchanged. Kidney injury molecule-1 tissue expression in raltegravir and dolutegravir groups was nonsignificantly elevated compared to control (1.2-fold compared to control). Renal histopathology by periodic acid–Schiff staining failed to reveal glomerular or tubular renal injury in any group.

Conclusion: These studies are consistent with integrase inhibitors competitively inhibiting creatinine secretion. While no evidence of direct nephrotoxicity was observed after two weeks of high-dose drug administration, additional studies may be performed to understand whether these drugs lead to chronic nephropathy.

Keywords

HIV, integrase inhibitor, Highly active antiretroviral therapy, drug interactions, nephrotoxicity

Introduction

The integrase inhibitors, raltegravir (RAL) and dolutegravir (DTG), are among the options for first-line antiretroviral therapy in HIV.¹ Integrase inhibitors have been shown to inhibit creatinine secretion, elevating serum creatinine without affecting glomerular filtration rate (GFR) in humans.² A recent clinical trial³ revealed that patients administered RAL had concomitant rises in serum cystatin C, a filtration marker unaffected by inhibition of secretion. In a mouse retroviral model, treatment with RAL led to increased autoimmunity manifested by hemolytic anemia and proteinuria.⁴ Given these findings, we sought to determine whether a two-week regimen of

¹Division of Nephrology, Indiana University School of Medicine, Indianapolis, USA

²Division of Clinical Pharmacology, Indiana University School of Medicine, Indianapolis, USA

³Division of Infectious Diseases, Indiana University School of Medicine, Indianapolis, USA

Corresponding author:

Michael T Eadon, Indiana University School of Medicine, Indianapolis, IN 46237, USA.
Email: meadon@iupui.edu

high-dose integrase inhibitor administration had a direct nephrotoxic effect in C57BL/6 mice.

Materials and Methods

RAL and DTG were dissolved in mouse drinking water at 0.2 and 0.014 mg/ml, respectively. In accordance with our Institutional Animal Care and Use Committee, eight-week-old C57BL/6 mice were then fed standard water (CTRL, $n=6$), RAL-containing water (40 mg/kg/day, $n=6$), or DTG-containing water (2.7 mg/kg/day, $n=6$) for two weeks and sacrificed. These doses correspond to approximately four-fold exposure over the standard human doses: RAL 400 mg twice daily and DTG 50 mg daily. The concentration of RAL in water, dose administered, and route of administration follow a previously published RAL mouse model.⁴ The concentration and dose for DTG were scaled to maintain a fourfold exposure as compared to the human daily dose. The two-week time point was selected as serum creatinine was reliably elevated at that time and modeled after a human study wherein two weeks of oral administration led to changes in creatinine secretion without affecting GFR.² Baseline and endpoint weight were measured and did not vary among CTRL, RAL, and DTG groups. Average daily water consumption did not vary between groups and was approximately 4.3 ml per mouse per day. Blood and urine were collected at baseline and upon sacrifice. Renal tissue was harvested as previously described.⁵

Endpoints were assessed including urine microalbumin by ELISA (Albuwell, Exocell), urine and serum creatinine by enzymatic assay (Diazyme DZ072B-K), blood urea nitrogen (BUN, Stanbio), plasma cystatin C by ELISA (Biovendor), renal histopathology with periodic acid–Schiff stain, renal macrophage infiltration by F480 immunohistochemistry (ab6640, Abcam), and plasma CD14 protein expression by immunoblot (SC-9150, Santa Cruz). Histopathology scoring, immunohistochemistry, and immunoblot were performed as previously described.⁵ Kidney injury molecule-1 (*HAVCR1*, KIM-1) gene expression was measured by real-time PCR of whole kidney and normalized to 18S expression as previously described.⁵ To ensure proper performance of the assays, positive control samples were selected for comparison. Plasma and renal tissue from C57BL/6 mice given lipopolysaccharide (LPS) endotoxin 5 mg/kg and sacrificed 24 h later were used as positive controls for the serum creatinine, BUN, cystatin C, KIM-1 expression, and F480 macrophage immunohistochemistry assays. Plasma of C57BL/6 mice sensitized (0.25 mg/kg) and reexposed (0.5 mg/kg) to LPS 24 h later was used as a positive control for plasma CD14 staining.⁶ Urine and renal

tissue from C57BL/6 mice given cisplatin 20 mg/kg and sacrificed 72 h later served as positive controls for the urine microalbumin and histopathology assays. Statistics were performed by Student's t-test for each group compared to the control group, as well as ANOVA between all groups.

Results

The results of our studies are not consistent with a direct nephrotoxic effect of the integrase inhibitors. Endpoint serum creatinine differed significantly between groups (ANOVA $p=0.0027$) and was elevated in both RAL (0.25 mg/dl, $p=0.03$) and DTG mice (0.30 mg/dl, $p=0.04$) as compared to control mice (0.17 mg/dl, Figure 1(a)). In contrast, BUN was unaffected (RAL = 17 mg/dl, DTG = 15 mg/dl versus CTRL = 19 mg/dl, Figure 1(b)). Plasma cystatin C did not vary significantly between groups (Figure 1(c)). KIM-1 gene expression of whole kidney was nonsignificantly elevated compared to control (1.2-fold elevation for both RAL and DTG, $p=0.14$ and 0.21, respectively). In contrast, positive control C57BL/6 mice sacrificed 24 h after LPS administration had elevated creatinine, BUN, cystatin C, and KIM-1 expression. Endpoint urine microalbumin was not elevated in the CTRL, RAL, or DTG groups with all microalbumin-to-creatinine ratios below a clinically significant threshold of 30 mcg/mg (Figure 1(e)). Positive control mice receiving cisplatin 20 mg/kg had elevated microalbumin to creatinine ratios.

In the RAL Switch trial,³ RAL-treated patients had greater decrement in sCD14, presumed secondary to reduced monocyte activation. To address this in our mice, CD14 plasma expression was measured (Figure 1(f) and (g)), but no discernable expression was found in the negative control (water), RAL, or DTG groups ($n=4$ per group, one of two blots shown). To ensure proper methodology, C57BL/6 mice sensitized and reexposed to LPS were used as a positive control because this model is known to express plasma CD14.⁶ Macrophage infiltration was also assessed by immunohistochemistry (Figure 2(a) to (e)) and no difference in number of macrophages per high power field was found in any group except the LPS positive control. Renal histopathology failed to reveal glomerular or tubular renal injury in any group, except the cisplatin control (Figure 2(f) to (j)).

Discussion

In this investigation, we sought to discern whether subtle nephrotoxicity from RAL and DTG would occur in concert with inhibition of creatinine secretion. Phase 1 clinical studies in humans revealed impairment

Figure 1. High-dose integrase inhibitors are not direct nephrotoxins in mice. (a) Serum creatinine was elevated in RAL and DTG mice, suggestive of inhibition of secretion (* = $p < 0.05$). No significant differences were noted between groups in BUN (b), cystatin C (c), renal KIM-1 expression (d), or urine microalbumin-to-creatinine ratios (e, dotted line is clinically significant threshold of 30 mcg/mg). Plasma CD14 (f-g) was not discernable in control, RAL, or DTG mice ($n = 4$ per group, one of two blots shown). Expression was normalized to a Ponceau stain and then expressed as a ratio to a positive control. The positive control was plasma of C57BL/6 mice sensitized and reexposed to lipopolysaccharide.⁶

of creatinine secretion after a two-week regimen of oral DTG. However, subtle findings of nephrotoxicity were observed in the Raltegravir Switch clinical trial and in a retroviral mouse model.⁴ The studies presented here do not support a direct nephrotoxic effect of integrase inhibitors in mice in the time frame examined. Serum creatinine levels of mice were reliably elevated after two weeks of RAL and DTG administration. However, highly sensitive injury markers measured in this study do not reveal evidence of renal injury after two weeks of high-dose drug administration.

Given the pharmacokinetic and pharmacodynamic profiles of these drugs, there is reason to believe that some of this study's findings might be extrapolated to humans. Most importantly, the drug exposure in the mice was sufficient to cause elevation in serum creatinine. Further, data obtained from the FDA (<http://www.accessdata.fda.gov/>) suggest the metabolism of these drugs is similar across species,^{7,8} without large differences in bioavailability, protein binding, and elimination. Both RAL and DTG are primarily metabolized through UGT1A1 glucuronidation.^{7,8} Although there is significant interspecies variation in CYP enzyme expression and activity,⁹ the UGT1A1 locus is well conserved between humans and mice.¹⁰ Murine in vivo

models have reasonably predicted human plasma clearance by glucuronidation for multiple drugs.¹¹ Specific to DTG, PK/PD studies have found the drug is extensively metabolized to an ether glucuronide in mice and eliminated through the hepatobiliary ducts,¹² analogous to the metabolism seen in human liver microsomes, humans, and other animals.^{13,14}

Although the time course for our investigation was selected to model a prior human DTG creatinine clearance study,² additional studies may need to be performed to understand whether these drugs lead to chronic nephropathy. From the Beck-Engeser et al. investigation, F1 NZBxNZW mice, a retroviral model of lupus disease, developed proteinuria and hemolytic anemia after 36–42 weeks of RAL exposure.⁴ However, mice without a retroviral background, including C57BL/6 mice, did not develop proteinuria or autoimmunity over this prolonged interval. In the RAL Switch trial,³ elevations of both serum creatinine and plasma cystatin C were observed in patients. While this finding provided an impetus for our study, we did not find an elevation of cystatin C in RAL and DTG mice. It is known that plasma cystatin C levels correlate with immune activation and inflammation in HIV patients.^{15,16} An alternative explanation for the RAL

Figure 2. Macrophage infiltration and histopathologic changes are not observed after integrase inhibitor administration. No increase in renal macrophages (a–e) or histopathologic changes (f–j) was found in RAL or DTG mice (Ten 40x images scored per mouse, $n = 6$). LPS exposed mice served as a positive control for macrophage infiltration and cisplatin exposed C57BL/6 mice served as the histologic positive control. (a) Neg Ctrl, (b) RAL, (c) DTG, (d) Pos Ctrl, (e) immunochemistry, (f) Neg Ctrl, (g) RAL, (h) DTG, (i) Pos Ctrl, (j) PAS histology.

Switch trial result is that RAL patients had inhibition of creatinine secretion as well as an elevation of cystatin C unrelated to GFR. After switching to RAL, these patients had a significant rise in plasma CD163, a marker of macrophage activation that may explain the concomitant rise in cystatin C.

Given the expectation of increased utilization of RAL and DTG as first-line antiretroviral therapy, we felt it was important to determine whether sensitive measures of nephrotoxicity might support a direct toxic effect of these drugs. An increase in serum creatinine may be secondary to either a drop in GFR or non-toxic inhibition of creatinine secretion through transporters such as OCT2. Given that the other interrogated toxic markers were not elevated, the elevation in serum creatinine observed is more consistent with benign inhibition of creatinine secretion.

Disclosure

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Declaration of Conflicting Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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