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

Short Communication – Antiviral Chemistry and Chemotherapy

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This is the author's manuscript of the article published in final edited form as:

Eadon, M. T., Zhang, H., Skaar, T. C., Hato, T., Dagher, P. C., Gupta, S. K., & Desta, Z. (2015). A two-week regimen of high-dose integrase inhibitors does not cause nephrotoxicity in mice. Antiviral Chemistry and Chemotherapy, 24(2), 72–76. https://doi.org/10.1177/2040206615595318

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 (RAL) containing water (40 mg/kg/day, n = 6), or dolutegravir (DTG) containing water (2.7 mg/kg/day, n=6) for 2 weeks and sacrificed. Endpoints were assessed including urine microalbumin, Kidney Injury Molecule-1 (KIM-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 RAL and DTG mice ($p < 0.05$) compared to control (RAL = 0.25 mg/dL, DTG = 0.30 mg/dL v. CTRL = 0.17 mg/dL). Blood urea nitrogen, cystatin C, and urine microalbumin were unchanged. KIM-1 tissue expression in RAL and DTG groups was non-significantly elevated compared to control (1.2-fold compared to control). Renal histopathology by PAS 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 2 weeks of high dose drug administration, additional studies may be performed to understand whether these drugs lead to chronic nephropathy.

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 trial [3] revealed that patients administered raltegravir had concomitant rises in serum cystatin C, a filtration marker unaffected by inhibition of secretion. In a mouse retroviral model, treatment with raltegravir led to increased autoimmunity manifested by hemolytic anemia and proteinuria [4]. Given these findings, we sought to determine whether a 2 week regimen of high dose integrase inhibitor administration had a direct nephrotoxic effect in C57BL/6 mice.

Methods:

Raltegravir and dolutegravir 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), raltegravir (RAL) containing water (40 mg/kg/day, n = 6), or dolutegravir (DTG) containing water (2.7 mg/kg/day, n=6) for 2 weeks and sacrificed. These doses correspond to approximately 4-fold exposure over the standard human doses: raltegravir 400 mg twice daily and dolutegravir 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 4-fold exposure as compared to the human daily dose. The 2 week time point was selected as serum creatinine was reliably elevated at that time and modeled after a human study wherein 2 weeks of oral administration led to changes in creatinine secretion without affecting glomerular filtration rate [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 (PAS), 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 re-exposed (0.5 mg/kg) to LPS 24 h later were 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 1A). In contrast, BUN was unaffected (RAL = 17 mg/dL, DTG = 15 mg/dL v. CTRL = 19 mg/dL, Figure 1B). Plasma cystatin C did not vary significantly between groups (Figure 1C). KIM-1 gene expression of whole kidney was non-significantly 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 1E). Positive control mice receiving cisplatin 20 mg/kg had elevated microalbumin to creatinine ratios.

In the Raltegravir Switch trial [3], raltegravir 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 1F and 1G), 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 re-exposed to lipopolysaccharide were used as a positive control because this model is known to express plasma CD14 [6]. Macrophage infiltration was also assessed by immunohistochemistry (Figure 2A-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 2F-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 of creatinine secretion after a 2 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 2 weeks of RAL and DTG administration. However, highly sensitive injury markers measured in this study do not reveal evidence of renal injury after 2 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/) suggests 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 dolutegravir, 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 raltegravir 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-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.

Acknowledgements: MTE was supported by the PhRMA foundation (Clinical Pharmacology Young Investigator Award) and the Norman S. Coplon Satellite Health Extramural Grant Program.

Disclosure: SKG received unrestricted educational grants from Merck & Co, Gilead Sciences, and Janssen Therapeutics. SKG has also received travel support from Bristol-Myers Squibb and Gilead Sciences to present research findings at scientific conferences. The other authors have nothing to disclose.

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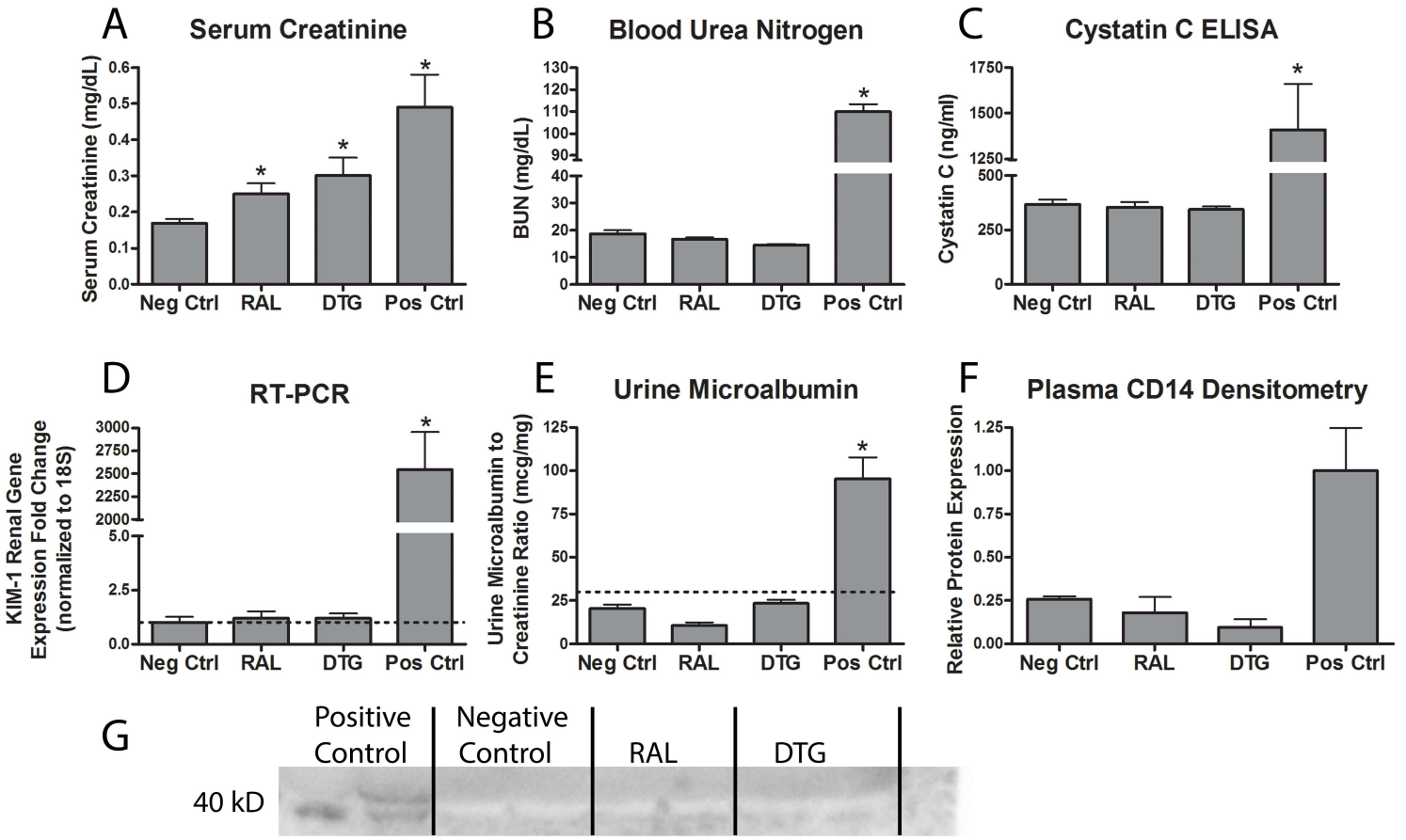
Figure 1: High-dose integrase inhibitors are not direct nephrotoxins in mice. (A) Serum creatinine was elevated in RAL and DTG mice, suggestive of inhibition of secretion (* = $p < 0.05$). No significant differences were noted between groups in BUN (B), cystatin C (C), renal KIM-1 expression (D), or 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 re-exposed to lipopolysaccharide [6].

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) were 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.

Figure 1


A
Serum Creatinine
Serum Creatinine (mg/dL)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Neg Ctrl
RAL
DTG
Pos Ctrl
B
Blood Urea Nitrogen
BUN (mg/dL)
0
10
20
30
40
90
100
110
120
130
C
Cystatin C ELISA
Cystatin C (ng/ml)
0
250
500
1250
1500
1750
D
RT-PCR
KIM-1 Renal Gene Expression Fold Change (normalized to 18S)
0.0
2.5
5.0
2000
2250
2500
2750
3000
E
Urine Microalbumin
Urine Microalbumin to Creatinine Ratio (mcg/mg)
0
25
50
75
100
125
F
Plasma CD14 Densitometry
Relative Protein Expression
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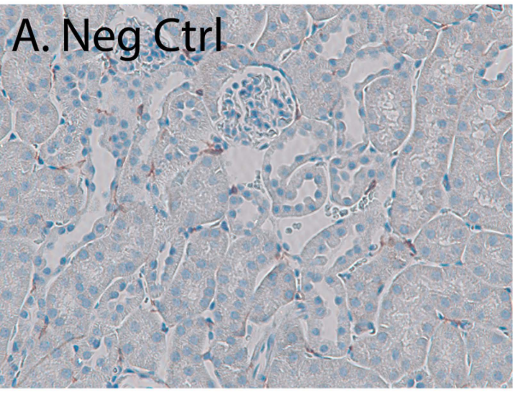
0.25
0.50
0.75
1.00
1.25
G
Positive Control
Negative Control
RAL
DTG
40 kD

Figure 2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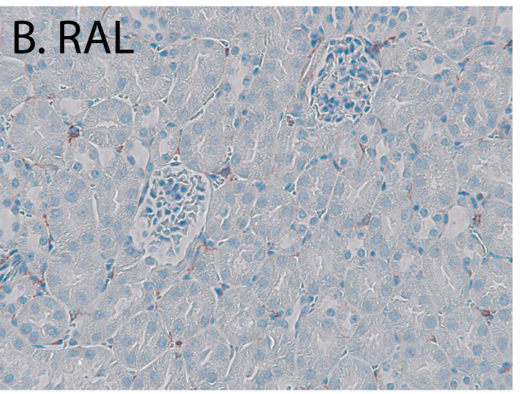
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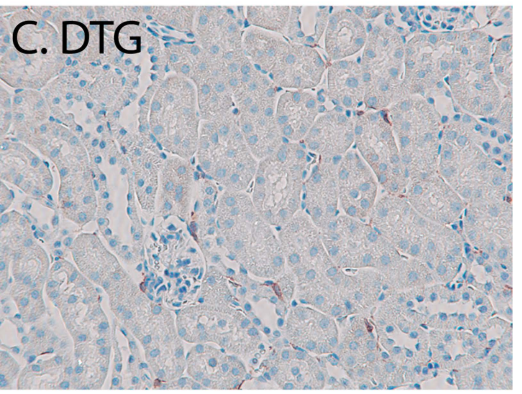
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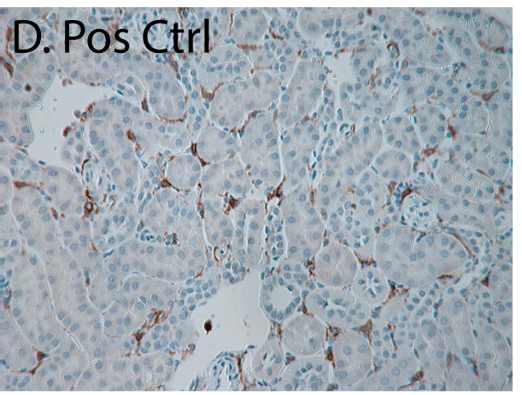
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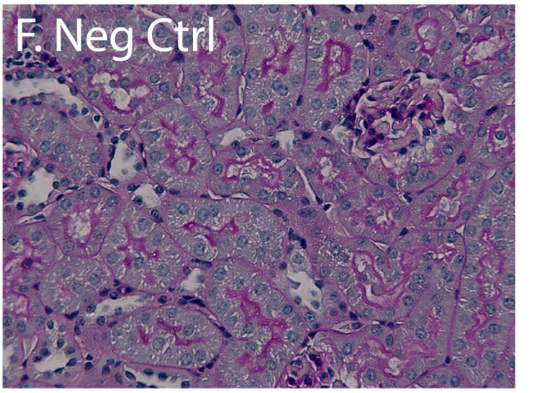
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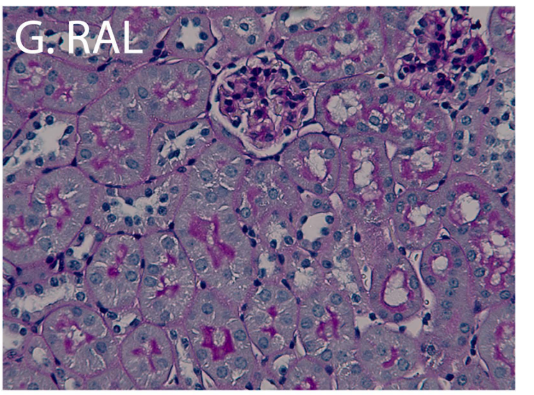
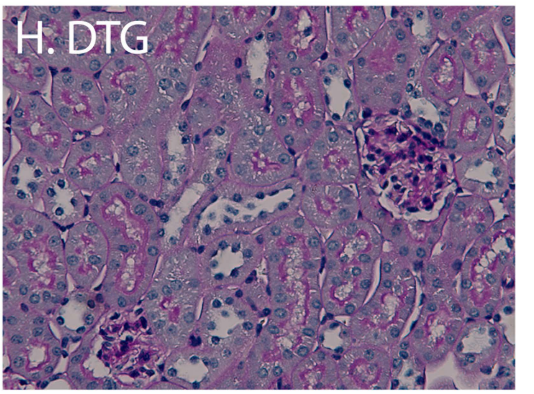

G. RAL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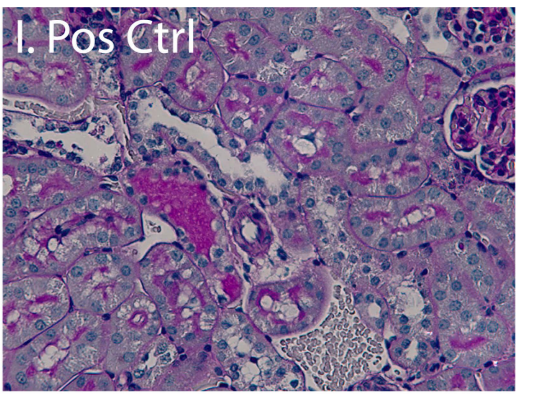
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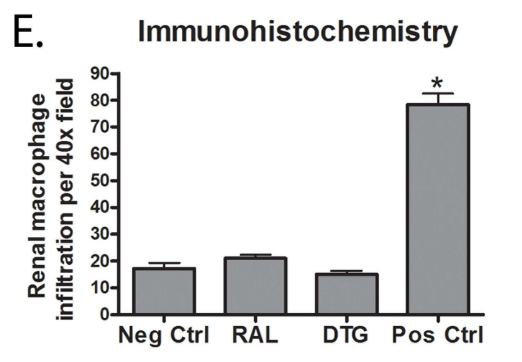
E.
Immunohistochemistry
Renal macrophage infiltration per 40x field
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80
70
60
50
40
30
20
10
0
*
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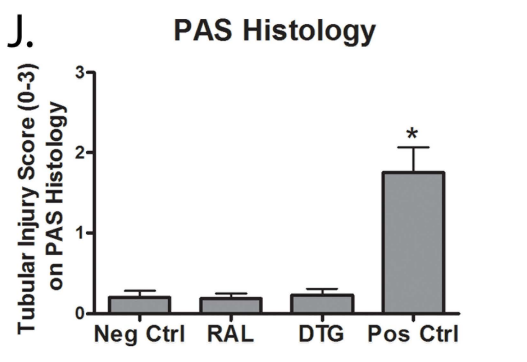
RAL
DTG
Pos Ctrl


J.
PAS Histology
Tubular Injury Score (0-3) on PAS Histology
3
2
1
0
*
Neg Ctrl
RAL
DTG
Pos Ctrl