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Expression of ATP-binding cassette membrane transporters in a HIV-1 transgenic rat model

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ABSTRACT

P-glycoprotein (P-gp, product of *Mdr1a* and *Mdr1b* genes), multidrug resistance associated proteins (Mrps), and breast cancer resistance protein (Bcrp), all members of the ATP-binding cassette (ABC) membrane-associated drug transporters superfamily, can significantly restrict the entry of antiretroviral drugs (ARVs) into organs which exhibit a barrier function such as the central nervous system (CNS) and the male genital tract (MGT). *In vitro*, HIV-1 viral proteins such as glycoprotein-120 (gp120) and transcriptional transactivator (tat) have been shown to alter the expression of these transporters and ARVs permeability. The objective of this study was to compare mRNA expression of these transporters, *in vivo*, in several tissues obtained from HIV-1 transgenic rats (Tg-rat) (8 and 24 weeks) with those of age-matched wild-type rats. At 24 weeks, significant changes in several drug transporter mRNA expressions were observed, in particular, in brain, kidney, liver and testes. These findings suggest that HIV-1 viral proteins can alter the expression of ABC drug transporters, *in vivo*, in the context of HIV-1 and further regulate ARVs permeability in several organs including the CNS and MGT, two sites which have been reported to display very low ARVs permeability in the clinic.

1. Introduction

Successful treatment of human immunodeficiency virus type-1 (HIV-1) infection requires antiretroviral drugs (ARVs) to reach effective therapeutic concentrations within the host's plasma and tissues. Despite the implementation of highly active antiretroviral therapy for the treatment of HIV infection and its success in eradicating viral load in the periphery, several tissues have been identified as viral sanctuary sites including the central nervous system (CNS), the male genital tract (MGT), gut-associated lymphatic tissues and renal epithelium [1,2]. These sites allow HIV replication to occur even in the presence of therapeutic concentrations of ARVs in plasma [3]. Furthermore, clinical studies have documented limited penetration of ARVs into several of these sites protected by unique blood–tissue barriers such as the blood–brain barrier (BBB) of the CNS [4] and the blood–testis barrier of the MGT [5].

One potential factor which may contribute to reduced tissue ARVs permeability is the expression of ATP-binding cassette (ABC) membrane-associated drug efflux transporters i.e., P-glycoprotein (P-gp), breast cancer resistance protein (Bcrp) and multidrug resistance associated proteins (Mrp), which can efflux extracellularly a wide range of substrates including many ARVs [6]. P-gp is encoded by *ABCB1* gene in humans and *abcb1a* and *abcb1b* genes in rodents and is expressed in many tissues including the brain (BBB), the MGT (blood–testis barrier), heart, liver, kidney and gastrointestinal tract [7]. P-gp is capable of transporting many ARVs i.e., protease inhibitors (PI), nucleoside/nucleotide reverse transcriptase inhibitors, (NRTI), integrase-strand transfer inhibitors and the CCR5 antagonist, maraviroc [6]. BCRP/Bcrp, encoded by *ABCG2* and *Abcg2* in humans and rodents, respectively, is also expressed at the BBB, the blood-testis barrier, kidney, liver and gastrointestinal tract [8] and can transport several NRTIs [9]. PIs have also been shown to inhibit both P-gp and Bcrp [10,11]. Encoded by *ABCC1* in humans and *Abcc1* in rodents, MRP1/Mrp1 is expressed in many tissues including lung, testis, kidney, skeletal and cardiac muscles, placenta and macrophages [12,13], and similar to P-gp, is capable of transporting many of the PIs [14]. MRP4/Mrp4 and MRP5/Mrp5 encoded by *ABCC4/Abcc4* and *ABCC5/Abcc5*

Abbreviations: ARVs, antiretroviral drugs; ABC, ATP-binding cassette; BBB, blood–brain barrier; Bcrp/BCRP, breast cancer resistance protein; CNS, central nervous system; GSH, glutathione; GSSG, glutathione disulfide gp120, HIV-1 glycoprotein-120; HIV, human immunodeficiency virus type-1; IL, interleukin; MGT, male genital tract; Mrps/MRPs, multidrug resistance associated proteins; NRTI, nucleoside reverse transcriptase inhibitor; P-gp, P-glycoprotein; PI, protease inhibitor; Tat, HIV-1 transcriptional transactivator; Tg-rats, HIV-1 transgenic rats; WT-rats, wild-type rats.

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respectively are predominantly cyclic nucleoside/nucleotide monophosphate transporters that are also capable of exporting several NRTIs such as abacavir, tenofovir and zidovudine [15,16]. Both transporters are known to be expressed in several tissues and cell-types including blood–brain and blood–testis barriers, kidney, hepatocytes and platelets [17]. The expression of P-gp, Bcrp, Mrp1, 4 and 5 could contribute to the reduced tissue concentrations of ARVs observed clinically in HIV infected patients [3].

Transgenic animal models are useful to study the role of viral proteins in HIV pathogenesis in tissues that are not directly infected with the actively-replicating virus. About a decade ago, Reid et al. developed a HIV transgenic rat (Tg-rat) model in which affected animals express a modified HIV transgene which has a functional deletion of the *gag* and *pol* genes that renders the virus non-infectious [18]. Due to the expression of the modified HIV transgene and subsequent expression of viral proteins, Tg-rats progressively develop immune abnormalities, cognitive and motor deficits, muscle wasting, cataracts, nephropathy and skin lesions that are remarkably similar to HIV infection [18]. Furthermore, these rats also have circulating levels of several HIV viral proteins [18] such as glycoprotein-120 (gp120) and trans-activator of transcription (tat) which have been previously shown by our group and others to regulate the expression of ABC transporters i.e., P-gp and Mrp1, in rodent and human astrocytes and brain microvessel endothelial cells, independent of active viral replication [19,20]. However, to the best of our knowledge, no studies have investigated the effect of the HIV transgene and viral proteins on ABC transporters mRNA expression *in vivo*. In this study, we examined the role of HIV viral proteins in the regulation of ABC transporter mRNA expression in several tissues obtained from two different ages of Tg-rats (8 and 24 weeks).

2. Methods

2.1. Animal model and tissue isolation

Male Sprague–Dawley Tg-rats and wild-type Sprague–Dawley rats (WT-rats) were purchased from Harlan Incorporated (Indianapolis, IN) at 6 weeks of age [18]. All rats were housed in pairs and provided with *ad libitum* food and water on a 12 h light/dark schedule. At 8 and 24 weeks of age, animals were anesthetized with 2-cc isoflurane and exsanguination was performed by cardiac puncture. Brain, heart, kidney, liver and testes were isolated and immediately frozen in liquid nitrogen. All animal protocols and procedures were approved by the University of Toronto and carried out in accordance with the University of Toronto Animal Care Committee and the Canadian Council on Animal Care.

2.2. Real-time quantitative RT-PCR

Total RNA was extracted from tissue samples of HIV Tg rats or WT-rats using TRIzol reagent according to manufacturer's instructions, and processed to cDNA as described previously [21]. Primer pairs were designed using Primer Express 3 Software (Applied Biosystems) and validated for specificity and efficacy using rat universal cDNA (see Supplemental Table S1). Real-time quantitative polymerase chain reaction (qPCR) was performed using SYBR Green Master Mix on an ABI 7900HTS Fast Real-Time qPCR System (Applied Biosystems, Foster City, CA). Relative expression of HIV *gp120* and HIV *tat* genes in each tissue was compared in Tg-rats between 8 and 24 weeks of age. ABC transporter expression in 8 and 24 weeks Tg-rats was compared to age-matched WT-rats using the delta–delta C_T method ($\Delta\Delta C_T$) [22]. The expression of each gene of interest in each tissue was calibrated and normalized to the expression of the housekeeping gene Cyclophilin B.

3. Results

3.1. Comparison of HIV viral gp120 and tat mRNA expression between the 8 and 24 weeks old Tg-rat groups

Applying qPCR analysis, HIV gp120 mRNA expression was detected in all the tissues obtained from the Tg-rats. We observed a significant increase in HIV gp120 mRNA expression in 24 week Tg-rat kidneys (3.42 ± 1.72-fold) and liver (5.76 ± 3.45-fold) compared to 8 week Tg-rats (Fig. 1A). Similar to HIV gp120, mRNA expression of HIV tat was observed in all the tissues tested with a trend in an increase in the expression at 24 weeks compared to the younger Tg-rat group (Fig. 1B).

3.2. Comparison of ABC transporters mRNA expression between WT-rats and Tg-rats at 8 and 24 weeks of age

To determine the effect of HIV viral transgene on ABC transporters mRNA expression, qPCR analysis was performed to identify mRNA fold changes for Mdr1a, Mdr1b, Mrp1, Mrp4, Mrp5 and Bcrp in Tg-rat tissues (brain, heart, kidney, liver and testes) and compared the results to the ones observed in tissues isolated from age-matched WT-rats. A fold change equal to 1 indicates no change, a fold change <1 indicates a decrease in mRNA expression and a fold-change >1 indicates an increase in mRNA expression. All Tg-rats mRNA expression data were compared to the relative mRNA expression of WT-rats within the same age group.

In 8 week Tg-rat brain tissue, we observed significantly lower Mrp1 mRNA expression (0.63 ± 0.18-fold) compared to age-matched WT-rats (1.00 ± 0.12). This significant decrease in Mrp1 mRNA expression persisted in the 24 week Tg-rat brain tissue (0.72 ± 0.10-fold) compared to WT-rats of the same age (1.00 ± 0.04). In addition, we also found significant decreases in Mdr1a (0.50 ± 0.05-fold), Mdr1b (0.69 ± 0.07-fold), Mrp4 (0.54 ± 0.05-fold) and Bcrp (0.48 ± 0.09) mRNA in Tg-rat brain tissue compared to WT-rat brain tissue of the same age (1.00 ± 0.05, 1.00 ± 0.11, 1.00 ± 0.06 and 1.00 ± 0.20, respectively). At 24 weeks of age, we observed a significant increase in brain Mrp5 mRNA expression in Tg-rat (1.37 ± 0.22-fold) compared to WT-rats brains of the same age (1.00 ± 0.04) (Table 1).

In 8 week old Tg-rat heart tissue, we found significantly lower Mrp1 mRNA expression (0.59 ± 0.33-fold) relative to WT-rats (1.00 ± 0.10) of the same age. Similarly, at 24 weeks of age, Tg-rat heart tissue showed significantly lower Mrp1 mRNA expression (0.80 ± 0.05-fold) relative to WT-rats of the same age (1.00 ± 0.09). In contrast, at 24 weeks of age, Tg-rats showed significant increases in heart Mdr1a (1.41 ± 0.09-fold), Mrp5 (1.27 ± 0.06-fold) and Bcrp mRNA (1.73 ± 0.44-fold) compared to age-matched WT-rats (1.00 ± 0.04, 1.00 ± 0.05 and 1.00 ± 0.05, respectively). We did not observe any significant changes in Mdr1b and Mrp4 mRNA expression in Tg-rat hearts at either 8 or 24-weeks of age when compared to age-matched WT-rats (Table 1).

In the Tg-rat kidneys, we detected a significant increase (1.59 ± 0.17-fold) in Mrp5 mRNA expression at 8 weeks of age when compared to age-matched WT-rats (1.00 ± 0.11). In contrast, at 24 weeks of age, we observed significant decreases in Mrp1 expression in kidney tissues (0.84 ± 0.08-fold), Mrp4 (0.59 ± 0.03-fold) and Mrp5 (0.47 ± 0.03-fold) compared to WT-rats of the same age. In the 24 week old Tg-rats both isoforms Mdr1a (1.85 ± 0.13-fold) and Mdr1b (1.52 ± 0.15-fold) as well as Bcrp (1.98 ± 0.32) kidney mRNA expression were significantly increased compared to age-matched WT-rats (Table 1).

In the 8 week Tg-rat liver tissue, we observed a significant decrease in Mrp4 mRNA expression (0.75 ± 0.08-fold) compared to age-matched WT-rats (1.00 ± 0.06). At 24-weeks, we found

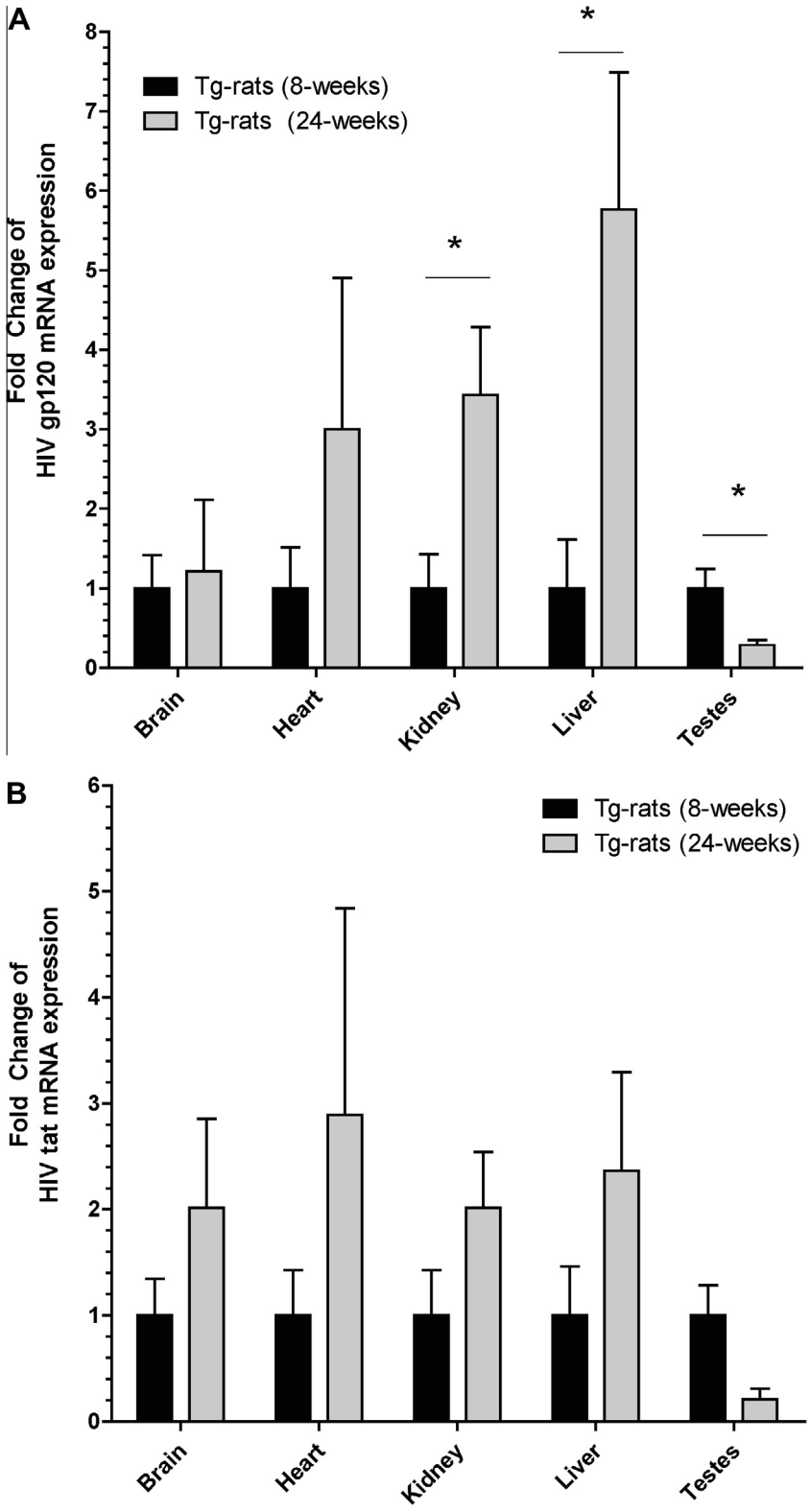

Fig. 1. Comparative mRNA relative expression of HIV viral transgenes in HIV Tg-rats. QPCR analysis was performed in 8 and 24 week old HIV Tg-rat tissues. Bars on the graph represent mean fold-change ± S.E.M. of (A) HIV-gp120 and (B) HIV-tat mRNA normalized to the house-keeping gene cyclophilin B, and relative to the 8-week HIV Tg-rat HIV gp120 and tat mRNA expression. Significant differences between 8- and 24-week old HIV Tg-rats are indicated by * ($P < 0.05$) and are determined using the Student's unpaired t test with Welch's correction ($n = 6$ animals per group).

significant decreases in Mdr1a (0.61 ± 0.03-fold) and Mrp1 (0.68 ± 0.04-fold) mRNA expression, and a significant increase in Mdr1b mRNA expression (1.42 ± 0.36-fold) compared to WT-rats of the same age (1.00 ± 0.04, 1.00 ± 0.11, and 1.00 ± 0.04, respectively). We did not observe any significant changes in Mrp5 and Bcrp mRNA expression at either 8 or 24 weeks of age in the Tg-rat liver tissues (Table 1).

In the Tg-rat testes, at 8 weeks of age, we detected a significant increase in Mrp1 mRNA expression (1.50 ± 0.10-fold) compared to WT-rats of the same age (1.00 ± 0.10). In the 24 week old Tg-rats

Table 1
ABC transporter mRNA mean fold-changes +/− SEM in HIV Tg-rats at 8 and 24 weeks of age relative to age-matched WT-rats.[a]

Age	Mdr1a	Mdr1b	Mrp1	Mrp4	Mrp5	Bcrp
Brain						
8 weeks						
WT-rats	1.00 ± 0.10	1.00 ± 0.16	1.00 ± 0.12	1.00 ± 0.10	1.00 ± 0.08	1.00 ± 0.12
Tg-rats	1.12 ± 0.11	0.92 ± 0.10	**0.63 ± 0.18***	0.83 ± 0.11	0.96 ± 0.05	0.98 ± 0.26
24 weeks						
WT-rats	1.00 ± 0.05	1.00 ± 0.11	1.00 ± 0.04	1.00 ± 0.06	1.00 ± 0.04	1.00 ± 0.20
Tg-rats	**0.50 ± 0.05***	**0.69 ± 0.07***	**0.72 ± 0.10***	**0.54 ± 0.05***	**1.37 ± 0.22***	**0.48 ± 0.09***
Heart						
8 weeks						
WT-rats	1.00 ± 0.15	1.00 ± 0.13	1.00 ± 0.10	1.00 ± 0.22	1.00 ± 0.11	1.00 ± 0.22
Tg-rats	0.75 ± 0.13	0.94 ± 0.22	**0.59 ± 0.33***	0.73 ± 0.14	0.82 ± 0.10	0.79 ± 0.10
24 weeks						
WT-rats	1.00 ± 0.04	1.00 ± 0.03	1.00 ± 0.09	1.00 ± 0.06	1.00 ± 0.05	1.00 ± 0.09
Tg-rats	**1.41 ± 0.09***	0.97 ± 0.09	0.80 ± 0.05	0.83 ± 0.08	**1.27 ± 0.06***	**1.73 ± 0.22***
Kidney						
8 weeks						
WT-rats	1.00 ± 0.08	1.00 ± 0.28	1.00 ± 0.12	1.00 ± 0.11	1.00 ± 0.11	1.00 ± 0.05
Tg-rats	1.03 ± 0.13	1.03 ± 0.21	0.86 ± 0.11	1.11 ± 0.14	**1.59 ± 0.17***	1.14 ± 0.07
24 weeks						
WT-rats	1.00 ± 0.06	1.00 ± 0.12	1.00 ± 0.04	1.00 ± 0.06	1.00 ± 0.09	1.00 ± 0.06
Tg-rats	**1.85 ± 0.13***	**1.52 ± 0.15***	**0.84 ± 0.08***	**0.59 ± 0.03***	**0.47 ± 0.03***	**1.98 ± 0.32***
Liver						
8 weeks						
WT-rats	1.00 ± 0.20	1.00 ± 0.13	1.00 ± 0.14	1.00 ± 0.06	1.00 ± 0.11	1.00 ± 0.27
Tg-rats	0.56 ± 0.04	1.42 ± 0.15	0.76 ± 0.16	**0.75 ± 0.08***	0.96 ± 0.14	0.62 ± 0.07
24 weeks						
WT-rats	1.00 ± 0.04	1.00 ± 0.04	1.00 ± 0.11	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.10
Tg-rats	**0.61 ± 0.03***	**1.23 ± 0.05***	**0.68 ± 0.04***	0.94 ± 0.13	0.94 ± 0.05	1.22 ± 0.15
Testis						
8 weeks						
WT-rats	1.00 ± 0.17	1.00 ± 0.17	1.00 ± 0.10	1.00 ± 0.14	1.00 ± 0.16	1.00 ± 0.20
Tg-rats	0.89 ± 0.08	1.15 ± 0.10	**1.50 ± 0.10***	1.03 ± 0.08	0.84 ± 0.06	1.09 ± 0.20
24 weeks						
WT-rats	1.00 ± 0.05	1.00 ± 0.06	1.00 ± 0.05	1.00 ± 0.04	1.00 ± 0.04	1.00 ± 0.28
Tg-rats	**2.25 ± 0.09***	**1.25 ± 0.04***	**0.82 ± 0.04***	**0.57 ± 0.03***	**2.40 ± 0.24***	0.61 ± 0.08

Significant differences between age-matched HIV Tg rat tissue and WT-rat tissue are indicated in bold.
[a] Results were calculated using the $\Delta\Delta C_T$ method.
* $p < 0.05$.

group, we observed significant increases in Mdr1a (2.25 ± 0.09-fold), Mdr1b (1.25 ± 0.04-fold) and Mrp5 (2.40 ± 0.24-fold) compared to age-matched WT-rats. Interestingly, in the 24 week Tg-rat testes, both Mrp1 (0.82 ± 0.04-fold) and Mrp4 (0.57 ± 0.03-fold) mRNA expression were significantly decreased relative to WT-rats testes of the same age. We did not observe any significant changes in Bcrp mRNA expression in testes tissues from either 8 or 24 week Tg-rats when compared to age-matched WT-rats (Table 1).

4. Discussion

The Tg-rat provides a unique animal model to investigate the effects of HIV-1 viral transgene on various pathologies related to HIV-1 infection including: chronic inflammation, increased oxidative stress and chronic immune dysfunction [18,23–25]. In this study, we report for the first time that the expression of the HIV viral transgene can significantly alter, *in vivo*, in an age-dependent and tissue-specific manner, the relative mRNA expression of ABC drug transporters i.e., P-gp, Mrps and Bcrp.

In the Tg-rat model by 24 weeks of age, we observed significant decreases in both brain mdr1a and mdr1b mRNA expression. One potential mechanism that HIV viral proteins can alter transporter expression is through activation of an inflammatory-mediated response. Our laboratory has previously demonstrated *in vitro,* that rodent and human astrocytes treated with HIV gp120 could release pro-inflammatory cytokines i.e., tumor-necrosis factor-α, interleukin (IL)-6, and IL-1β, and that subsequent exposure to IL-6 or HIV gp120 decreased P-gp expression and function significantly [20,26]. Previous studies with Tg-rats have also demonstrated similar increases in pro-inflammatory cytokines [23], which further supports their potential role in the regulation of ABC drug efflux transporters within this model.

At present, to the best of our knowledge, no studies have examined the effect of HIV viral proteins on the regulation of ABC drug transporters at the level of the testes, heart, liver or kidneys. In Tg-rats, we observed that both testes and heart appeared to be affected differently compared to the brain, showing a significant increase in mRNA expression of mdr1a and mdr1b, and Mrp5. A study by Jorajura et al. demonstrated that active HIV replication leads to significant increases in Mrp1 and Mrp5 mRNA expression in human macrophages [27]. Furthermore, at 8 weeks of age, we observed a significant increase in Mrp1 mRNA expression in testes of Tg-rat, while a significant decrease was detected in the Tg-rat heart. Taken together these findings suggest that changes in ABC drug transporter mRNA expression appear to be not only tissue-specific, but also potentially related to changes in HIV transgene expression.

In the Tg-rat heart tissue, we observed significant increases in Mdr1a (P-gp), Mrp5 and Bcrp mRNA expression at 24 weeks of age. Several studies examining heart disease in HIV infected patients have shown increase cardiovascular risk and arterial inflammation when compared to healthy uninfected subjects [28]. A study by Dazert et al. found a similar increase in mRNA and protein

expression of MRP5 in ischemic human heart tissue when compared to non-failing heart controls [29]. Another study demonstrated significant increases in BCRP mRNA expression in human cardiomyopathic hearts tissues compared to tissue controls [30]. In addition, Bcrp expression is demonstrated to be increased under ischemic conditions [31]. Although the exact mechanism by which Bcrp may be protective against hypoxia is not known; it has been suggested that Bcrp can reduce the accumulation of heme and porphyrin which are cytotoxic in excess concentrations [31]. Taken together, these findings suggest that Mrp5 and Bcrp may be involved in protecting the heart from increased inflammation and oxidative stress which occur during heart failure and HIV infection.

The liver is not only the primary organ involved in drug metabolism, but also expresses several ABC drug transporters involved in the disposition of ARVs including the HIV PIs and NNRTIs [32]. In this study, we observed an increase in HIV gp120 expression in 24-weeks Tg-rat liver compared to 8-weeks of age, and significant decreases in the mRNA expression of Mdr1a and Mrp1. In contrast, we observed a significant increase in mRNA expression of Mdr1b by 24-weeks of age in Tg-rats. Previous studies examining the effect of lipopolysaccharide-induced inflammation on Mdr1a and Mdr1b liver mRNA expression in rodents have reported similar results [33,34]. These findings suggest that changes in Mdr1 and Mrp1 mRNA expression in liver could affect the hepatobiliary clearance of several ARVs.

In the Tg-rat kidney tissue, at 24 weeks of age, we observed a significant decrease in mRNA expression of Mrp4. Tenofovir, a preferred NRTI used extensively in ARV therapy, is primarily eliminated through glomerular filtration and tubular secretion [35], and has been identified as a substrate of Mrp4 [15]. A study by Kohler et al. demonstrated that $Mrp4^{-/-}$ knockout mice were more susceptible to tenofovir-mediated mitochondrial toxicity due to the increased drug intracellular concentrations [36]. Taken together, these findings suggest that a decrease in Mrp4 mRNA expression could affect tenofovir elimination in the renal proximal tubules and lead to an increased risk of nephrotoxicity [15,36].

Another mechanism by which HIV viral proteins can affect the expression of ABC transporters is through oxidative stress [37]. Evidence from previous clinical studies suggest that HIV infected patients develop chronic oxidative stress characterized by higher blood plasma concentrations of hydroperoxides and malondialdehyde, markers which are indicative of polyunsaturated fatty acid peroxidation [38,39]. The cellular response to oxidative stress involves the antioxidant glutathione (GSH), which acts as reactive oxygen species (ROS) scavenger. During cell injury, GSH is oxidized to glutathione disulfide (GSSG) which can be exported by several Mrp isoforms in particular, Mrp1 [40]. A decrease in the brain, kidney and liver expression of Mrp1 and Mrp4 in 24 weeks old Tg-rats may affect the ability to export cellular GSH and GSSG. Kline et al. have previously demonstrated that Tg-rats have decreased vascular nitric oxide and elevated vascular levels of superoxide radicals [24]. Our laboratory has demonstrated that HIV gp120 can induce oxidative stress in primary cultures of rat astrocytes [41]. However, in contrast to the observed decrease in Mrp1 mRNA expression in 24-week old HIV Tg-rat brain, our laboratory has previously reported a significant increase in Mrp1 expression in primary cultures of rat astrocytes treated with HIV-1 gp120, resulting in enhanced export of GSH and GSSG [41].

Within the context of HIV-infection, the regulation of drug transporters is complex and dependent on activation of the immune system which results in inflammation and oxidative stress. Our study provides *in vivo* evidence that HIV viral transgene can affect the expression of ABC drug transporters in a tissue-specific manner. Changes in transporter expression in the brain and the testes may result in alterations in the permeability and tissue distribution of ARVs that are known to be substrates of several of these [42]. Further studies are needed to determine which HIV viral proteins can affect, *in vivo*, the regulation of ABC drug transporters in specific tissues, and how potential changes in transporter expression could ultimately affect ARVs distribution and permeability.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.092.

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