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In vivo examination of hydroxyurea and the novel ribonucleotide reductase inhibitors trimidox and didox in combination with the reverse transcriptase inhibitor abacavir: suppression of retrovirus-induced immunodeficiency disease

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Abstract

Inhibition of ribonucleotide reductase (RR) has gained attention as a potential strategy for HIV-1 therapy through the success of hydroxyurea (HU) to potentiate the activity of the nucleoside reverse transcriptase inhibitor (NRTI) didanosine (ddI) in clinical trials. However, the use of HU has been limited by its development of hematopoietic toxicity. In this study, the novel RR inhibitors didox (DX; 3,4-dihydroxybenzohydroxamic acid), and trimidox (TX; 3,4,5-trihydroxybenzamidoxime) were evaluated along with HU for anti-retroviral efficacy in LPBM5-induced retro-viral disease (MAIDS) both as monotherapeutic regimens and in combination with the guanine containing NRTI abacavir (ABC). Anti-retroviral drug efficacy was determined by measuring inhibition of splenomegaly, hypergammaglobulinemia, and splenic levels of proviral DNA. In this study, all RRIs tested showed the ability to improve the efficacy of ABC in the MAIDS model by reducing splenomegaly, hypergammaglobulinemia, and splenic proviral DNA levels.

Keywords: Ribonucleotide reductase; Murine AIDS; Hydroxyurea; Trimidox; Didox; Abacavir

1. Introduction

The need for alternative therapies for HIV infection has been highlighted by the demonstration of the development of multi-drug resistant HIV-1 strains (Shafer et al., 1998), metabolic abnormalities (Vigouroux et al., 1999), and the demonstration of low-level viral replication (Dornadula et al., 1999) in patients receiving highly active anti-retroviral therapy (HAART). These problems are further compounded by complicated drug administration schedules, often resulting in poor compliance, and by the high cost of therapy.

One alternative therapeutic strategy that has received attention is the use of ribonucleotide reductase inhibitors (RRI). Ribonucleotide reductase (RR) catalyses the formation of deoxyribonucleotides from the corresponding ribonucleotides, and represents the rate-limiting step in the production of DNA precursors (Reichard, 1993). Viruses, including HIV, are completely lacking in their ability to synthesize dNTPs, and are therefore completely dependent upon their host cells for production of these essential substances. Selective depletion of host cell dNTP pools without irreversibly impairing host cell dNTP synthesis is an intriguing target for inhibiting HIV replication (Mills et al., 1992a,b).

The RR inhibitor HU has been the primary focus of this strategy, since it is available for therapeutic clinical use, successfully inhibiting in vitro HIV-1 proviral DNA synthesis (Gao et al., 1994), and potentiating the anti-retroviral activity of several dideoxynucleoside (ddN) analogs (Gao et al., 1994; Malley et al., 1994; Lori et al., 1994). In addition, hydroxyurea has been shown to potentiate the anti-herpesvirus activity of several ddN analogs (Neyts and De Clerq, 1999).

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Clinical studies have supported the in vitro observations that HU can successfully potentiate the activity of ddI (Lori et al., 1997a,b), or ddI and d4T (Rutschmann et al., 2000; Galpin et al., 1998) in HIV-infected patients. It has also been shown that regimens containing a protease inhibitor, an NRTI, and HU are successful in reducing viral load to undetectable levels, decrease the number of activated CD8+ cells, and increase the number of CD4+ cells (Lori et al., 1999a,b).

The widespread use of HU in HIV-infected patients is still somewhat controversial due to its notorious ability to induce significant hematological disturbance (Maserati, 1999). In patients with advanced disease or in those receiving other anti-retroviral drugs that suppress hematopoiesis, the bone marrow suppression associated with HU may be further exacerbated (Foli et al., 1997). In addition to these problems, the rapid plasma clearance of HU (Moore and Hurlbert, 1985) and its relatively weak in vitro enzyme inhibition (Elford, 1968) may limit the in vivo RR inhibitory activity of HU. In an attempt to achieve greater RR inhibition than HU, several more potent RR inhibitors have been synthesized (van't Riet et al., 1979). Two of these compounds TX and DX were found to be particularly potent in vitro RR inhibitors (Elford and van't Riet, 1985). The structures of TX, DX, and HU are shown in Fig. 1.

A number of differences have been observed between the novel RRIs DX and TX in comparison to HU. Potentially the most important difference from a clinical perspective may be the effects DX and TX have upon dNTP levels compared to HU (Elford and van't Riet, 1985). HU has been consistently shown to reduce only the purine dNTP pools (dATP and dGTP) in various cell lines (Snyder et al., 1984; Bianchi et al., 1986; Gao et al., 1998). In contrast, DX and TX have been shown to reduce pools of both purine and pyrimidine dNTPs (Elford et al., 1980; Szekeres et al., 1994a; Elford and van't Riet, 1985). These findings may prove to be of clinical importance by allowing a larger number of therapeutic options in treatment of human AIDS.

Additional studies have shown that TX and DX have more potent in vivo anti-tumor activity than HU in several murine tumor models (Szekeres et al., 1994a,b; Elford et al., 1979). Results from our laboratory and others have shown that TX and DX have effective anti-retroviral activity both alone and in combination with ddI in the MAIDS model (Mayhew et al., 1997), HIV-infected HuPBMC SCID model (Ussery et al., 1999), and the Rauscher murine retrovirus model (Mills et al., 1992a,b). Importantly, it has also been demonstrated that TX and DX are less toxic to normal human and murine hematopoietic progenitor cells when compared to HU in vitro and in vivo (Mayhew et al., 1999, 2002b).

MAIDS is induced by inoculation with a complex of retroviruses called LPBM5 murine leukemia virus (MuLV), originally recovered from a radiation-induced lymphoma of C57BL/6 (B6) mice (Laterjet and Duplan, 1962). Many of the features of MAIDS are similar to those of HIV. Some of these similarities include development of a profound immunodeficiency characterized by deficits in B- and T-lymphocyte function as well as deficiencies in macrophage function. Early stage disease is characterized by polyclonal activation of lymphocytes and proliferation associated with progressive lymphadenopathy and splenomegaly (Chattopadhyay et al., 1991; Jolicoeur, 1991). Advanced stages of the disease are associated with profound immunodeficiency and enhanced susceptibility to opportunistic infections (Doherty et al., 1995) and development of secondary neoplasms, especially B-cell lymphomas (Buller et al., 1987).

Despite the similarities between HIV and MAIDS, there are several important differences. The major cellular targets for LPBM5 MuLV infection are B-lymphocytes, and not CD4 T cells as in HIV. LPBM5 MuLV is also much simpler in structure than HIV, lacking the tat, rev, and nef regulatory genes (Magnani et al., 1997). Also, the cause of death in MAIDS is believed to be severe pulmonary compromise secondary to lymphoid infiltration and enlarged thoracic lymph nodes (Jolicoeur, 1991).

MAIDS has been widely used as a model to evaluate experimental anti-HIV-1 compounds (Suruga et al., 1998; Fraternale et al., 2002; Magnani et al., 1997; Mayhew et al., 2002a). A particular advantage with the use of MAIDS for evaluation of experimental anti-retroviral compounds is that, unlike other murine models of retrovirus infection, the disease progresses over a substantial period of time. This permits administration of experimental drugs for several months, and evaluations of therapeutic benefit versus toxicity can be made in infected animals. Because B-cell proliferation is a major component of LPBM5 MuLV infection, MAIDS provides a good model for the preclinical study of compounds such as HU that have been proposed to be beneficial in HIV infection due in part to their cytostatic properties (Lori and Lisziewicz, 1998).

It has previously been demonstrated by our laboratory that the RRIs TX, DX, and HU serve as effective treatments for LPBM5 MuLV infection alone (Mayhew et al., 1997, 1999),

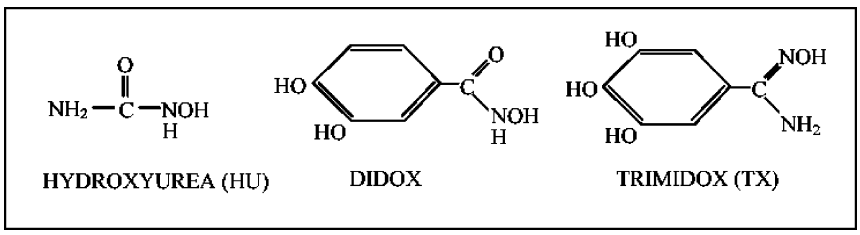

Fig. 1. Structure of RRIs.

or in combination with ddI (Mayhew et al., 1997). It has also been demonstrated that HU, TX, and DX can safely be administered to MAIDS infected mice for over 1 year (Mayhew et al., 1997). Further comparisons of TX and DX with HU have shown that TX and DX are less toxic to normal and infected bone marrow than HU (Mayhew et al., 1997). Most recently it has been demonstrated that short-term treatment with TX and DX is able to reverse late-stage MAIDS disease (Mayhew et al., 2002a). The purpose of the described studies was to further compare in vivo the ability of hydroxyurea and the novel ribonucleotide reductase inhibitors DX and TX to suppress retroviral infection in the MAIDS model when combined with the NRTI compound abacavir.

2. Materials and methods

2.1. Mice

Female C57BL/6 mice aged 8–10 weeks were purchased from Charles River/NCI (Bethesda, MD, USA), and were housed in micro-isolator cages in a temperature and humidity-controlled environment. Mice were fed Purina Lab Chow and water ad libitum. The experimental animal protocol used in these studies was approved by the University of Kentucky IACUC committee.

2.2. Infection of mice with LPBM5 MuLV

The G6 subclone of chronically LPBM5 MuLV-infected SC-1 cells was kindly provided by Dr. Donald Cohen, Department of Microbiology and Immunology, University of Kentucky. G6 cells were grown in Dulbecco's Modified Eagle Medium (Gibco, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (Sigma Chemical Co., St. Louis, MO, USA), and 1% penicillin–streptomycin (Gibco). Virus used for inoculation of mice was prepared by removal of the cell supernatant. The cell monolayer was subsequently lysed by repeated freeze–thaw cycles to yield intra-cellular virus (Mayhew et al., 1997, 2002a,b). This was combined with the cell supernatant, centrifuged at 300 × *g* for 10 min and 0.45 μm filtered. Inoculation of virus was performed by two separate 0.5 ml i.p. injections 3 days apart, per mouse.

2.3. Treatment of LPBM5 MuLV-infected mice

LPBM5-infected animals were randomly assigned to groups of eight to receive the various treatment regimens (see Table 1). Doses of RRIs were chosen based upon concentrations, which had previously been shown by our laboratory to enhance the activity of ddI (Mayhew et al., 1997), and doses of ABC were chosen based upon the manufacturer's recommendations. TX and DX were provided by Dr. Howard Elford (Molecules for Health Inc., Richmond, VA, USA). HU was purchased from Sigma Chemical Co., and ABC was provided by Dr. Trevor Scott (Glaxo Smith Kline, Research Triangle Park, NC, USA).

Table 1
Experimental groupings

Normal control	Infected + TX150
Infected control (MC)	Infected + TX150 + ABC100
Infected + HU60	Infected + TX150 + ABC200
Infected + HU60 + ABC200	Infected + DX300
Infected + HU150	Infected + DX300 + ABC200
Infected + HU150 + ABC200	Infected + DX350
Infected + HU200	Infected + ABC200

All doses of drugs are in milligram per kilogram of body weight.

All mice in groups listed above received the listed drug doses in milligram per kilogram body weight. In addition to the groups listed above, 16 non-infected, non-treated animals served as normal controls, and 16 LPBM5-infected, non-treated animals served as MAIDS controls. Drug treatment began 7 days after the first inoculation of virus, and continued daily for 8 weeks. HU, TX, DX, and ABC were injected i.p. in a final volume of 0.5 ml for each drug. Groups receiving combination therapy were given a total of two injections per day (0.5 ml of each drug). Drugs were prepared weekly, sterile filtered, and stored at 4 °C. Drugs were warmed to body temperature prior to injection.

2.4. Procurement of tissues for analysis of anti-viral drug efficacy and hematopoietic toxicity

After 4 and 8 weeks of treatment, four mice from each group were randomly selected and sacrificed for analysis of anti-viral drug efficacy. Blood was collected from each mouse by cardiac puncture, and complete blood counts were performed using a Baker 9110 +CP (ABX Diagnostics, Irvine, CA, USA). Serum was obtained by centrifugation of blood at 10,000 × *g* for 10 min and was frozen at −20 °C until used. The spleens were rapidly removed, weighed, and cut approximately in half. One half was immediately frozen in a dry-ice/ethanol bath and stored at −80 °C until used for DNA extraction.

2.5. Enzyme-linked immunosorbant assay for murine IgG

Serum was obtained after 4 and 8 weeks as described above and stored at −20 °C until used. A standard enzyme-linked immunosorbent assay technique (ELISA) was used to quantify levels of murine IgG. Ninety-six-well microtiter plates were coated overnight at 4 °C with 1.5 μg/ml goat anti-mouse IgG (Fc-chain-specific; Jackson ImmunoResearch, West Grove, PA, USA) in 0.1 M $NaHCO_3$. After washing and blocking with 0.1% bovine serum albumin (Sigma) in PBS, diluted serum samples and serially diluted murine IgG standards (Sigma) of known concentrations were added to plates and incubated

at room temperature for 2 h. After washing, 0.3 μg/ml alkaline phosphatase-conjugated anti-mouse IgG (Jackson ImmunoResearch) in PBS was added and incubated at room temp for 1 h. After washing, *p*-nitrophenyl phosphate substrate (Sigma) was added and incubated for 15 min at 37 °C. The reaction was stopped by addition of 3 M NaOH and the absorbance of each well was read at 405 nm. Serum IgG concentrations were determined by comparison with the murine IgG standards.

2.6. Extraction of genomic DNA

Genomic DNA was extracted from three spleens from each experimental group using the DNeasy tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Spleen size was not taken into account when selecting tissue for DNA extraction. DNA was eluted from spin columns and stored at −20 °C until used in PCR reactions. The yield and purity of extracted DNA was quantified spectrophotometrically.

2.7. Semi-quantitative PCR for integrated proviral DNA

The following primers, specific for the p12 gag region of the LPBM5 defective virus (BM5-def) genome were used for PCR amplification: 5′-CCT TTT CCT TTA TCG ACA CT-3′ (sense), and 5′-ACC AGG GGG GGA ATA CCT CG-3′ (anti-sense). The expected size of the amplified product was 246 base pairs. A second pair of oligonucleotide primers, designed using the Oligo (version 6.15) computer software (Molecular Biology Insights Inc., Cascade, CO, USA) were used to amplify a region of the murine B-actin gene which served as an endogenous control. The B-actin primer sequences are: 5′-CAC TGT GCC CAT CTA CGA-3′ (sense), and 5′-ACA GGA TTC CAT ACC CAA G-3′ (anti-sense) and amplified a product of 334 base pairs. PCR amplification in the linear range was performed in a final volume of 25 μl, containing 0.25 μg genomic DNA, 1 pg of each primer and a 1× solution of AmpliTaq gold master mix (Applied Biosystems, Branchburg, NJ, USA). BM5def was amplified by hot-start and initial denaturation at 95 °C for 10 min followed by 22 cycles of denaturation at 95 °C for 1 min, annealing at 57 °C for 1.5 min and extension at 72 °C for 2 min, followed by a final extension at 72 °C for 8 min. β-Actin was amplified by hot-start and initial denaturation at 95 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 45 s, and extension at 72 °C for 45 s followed by a final extension at 72 °C for 10 min. For quantification, gels were stained with ethidium bromide and densitometry was performed using 1D Image Analysis Software (Kodak, Rochester, NY, USA). Each BM5def band was normalized to its corresponding G6PDH band before comparison between experimental groups, and each data point represents the mean and S.D. for a minimum of three mice per experimental group.

2.8. Assay of bone marrow progenitors

Contents of femurs were flushed in ice cold PBS with a 22-gauge needle. Single cell suspensions of bone marrow were made by gentle flushing through an 18-gauge needle, and bone marrow from each experimental group was pooled for analysis of committed granulocyte/macrophage (CFU-GM), and erythroid (BFU-E) progenitors, according to previously published methods (Mayhew et al., 1997). Briefly, 25,000 bone marrow cells were plated in duplicate in 1ml methylcellulose medium (Stem Cell Technologies, Vancouver, Canada) containing 1% methylcellulose in Iscove's minimal Dulbecco's media, 15% FBS, 1% bovine serum albumin, 10 μg bovine pancreatic insulin, 200 μg/ml human transferrin (iron saturated), 10^{-4} M β-mercaptoethanol, 2 mM L-glutamine, 50 ng/ml recombinant murine (rm) stem cell factor, 10 ng/ml recombinant human (rh) interleukin-6, and 3 units/ml rh-erythropoietin. After vortexing, duplicate samples were plated in six-well tissue culture plates and incubated at 37 °C in air containing 5% CO_2 for 10 days. Using an inverted microscope, CFU-GM and BFU-E were identified based on their morphology, and colonies containing greater than 50 cells were scored.

2.9. Statistical analysis

The Student's two-tailed *t*-test was used to determine the significance of differences between groups. A *P*-value of <0.05 was considered to be significant.

3. Results

3.1. Inhibition of splenomegaly

Infection of mice with LPBM5 MuLV results in immunodeficiency disease and lymphoproliferation characterized by splenomegaly (Morse et al., 1992). In this study all infected control animals developed extensive peripheral lymphadenopathy and splenomegaly. Spleen weights from each experimental group are shown in Fig. 2. By the end of the eighth week of infection, spleens of infected controls weighed approximately six- to seven-fold higher than non-infected controls. ABC monotherapy showed only a modest reduction in spleen weight versus MAIDS controls (MC) (approximately 29%). Combination of ABC with RRIs was able to greatly improve the activity level of ABC, reducing the increase in spleen weight due to MAIDS infection by: 86% for HU150 + ABC200 and approximately 90% for TX150 + ABC200; 91% for DX300 + ABC200. The combination treatment group spleen weights were only slightly different from normal control values. The best anti-viral activity, however, was demonstrated by DX350 monotherapy, which was not significantly different from non-infected control values ($P = 0.347$), but was significantly different from MC ($P < 0.005$).

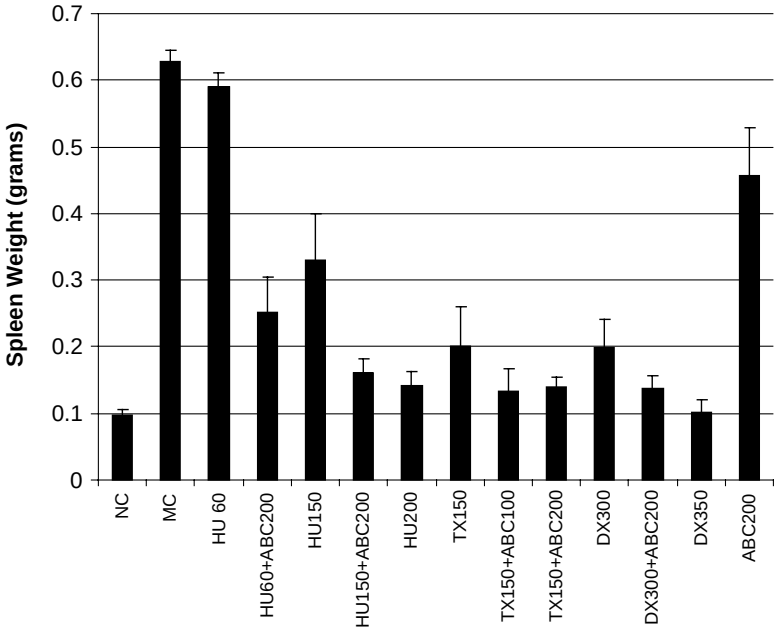

Fig. 2. Effect of drug treatment on inhibition of viral-induced splenomegaly in LPBM5 MuLV-infected mice. LPBM5 MuLV-infected animals were treated daily for 8 weeks with RRIs alone or in combination with ABC. After 8 weeks of drug treatment, animals were sacrificed and their spleens were weighed. Bars represent the mean and standard deviation of at least three animals per group.

3.2. Inhibition of hypergammaglobulinemia

Early progression of MAIDS is associated with polyclonal activation of B-cells resulting in increased serum immunoglobulin concentrations (Klinman and Morse, 1989). Mean serum IgG concentrations for each experimental group are shown in Fig. 3. By the end of the eighth week of drug treatment, hypergammaglobulinemia was evident in infected controls, which showed a six- to seven-fold increase in IgG levels versus non-infected controls. All drug treatment groups were effective in significantly lowering the level of serum immunoglobulin versus infected control, with the exception of the lowest monotherapeutic dose of HU. ABC200 showed approximately 45% inhibition, with all HU and TX regimens showing comparable results. The most effective treatment at inhibiting the increase in serum IgG level was in the DX300 + ABC200 group. This group showed no increase in IgG levels versus non-infected controls.

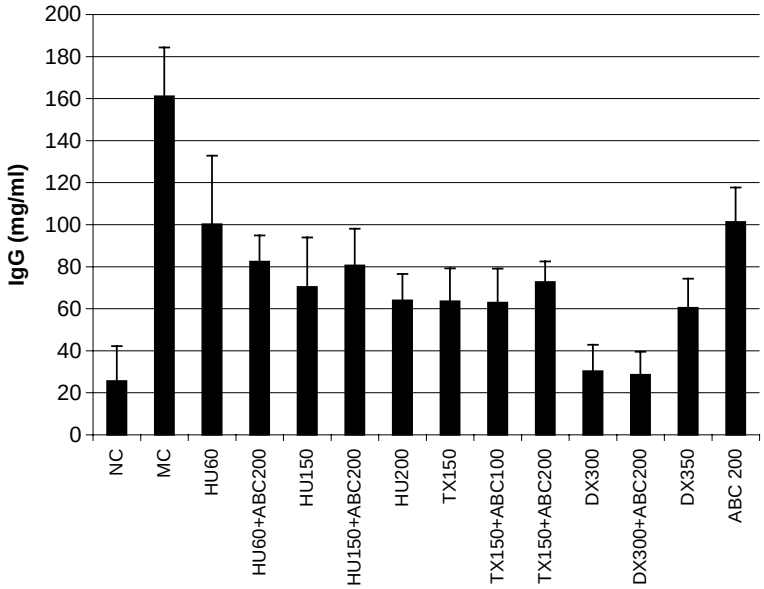

Fig. 3. Effect of drug treatment on inhibition of viral-induced hypergammaglobulinemia in LPBM5 MuLV-infected mice. LPBM5 MuLV-infected animals were treated daily for 8 weeks with RRIs alone or in combination with ABC. After 8 weeks of drug treatment, animals were sacrificed and their serum IgG levels were measured as described in the Section 2. Bars represent the mean and standard deviation of at least three animals per group.

DX300 monotherapy showed similar results, with a slightly higher mean value and slightly more variation within the group.

3.3. Inhibition of LPBM5 proviral DNA

Relative levels of splenic BM5 def proviral DNA were evaluated by semi-quantitative PCR (Fig. 4A and B). After 8 weeks of drug treatment, all drug treated groups showed reduced levels of pro-viral DNA compared to infected controls.

3.4. Gross toxicity

There was no evidence of gross toxicity in any of the treatment groups presented in this paper, however, when HU200 mg/kg was combined with ABC, fatal toxicity was observed in a high percentage of animals receiving these regimens (data not shown). This is in contrast to TX and DX, which were well tolerated at relatively high doses both alone and in combinations with ABC.

3.5. Effects on peripheral blood indices

Complete blood counts were performed after 8 weeks of drug treatment to monitor the effects of drug treatment on peripheral blood indices. Although no statistically significant changes in white blood count (WBC) values were observed, there was a general trend present indicating that groups treated with HU had the lowest mean WBC, followed by DX, then TX. ABC treatment did not tend to alter WBC appreciably (Fig. 5).

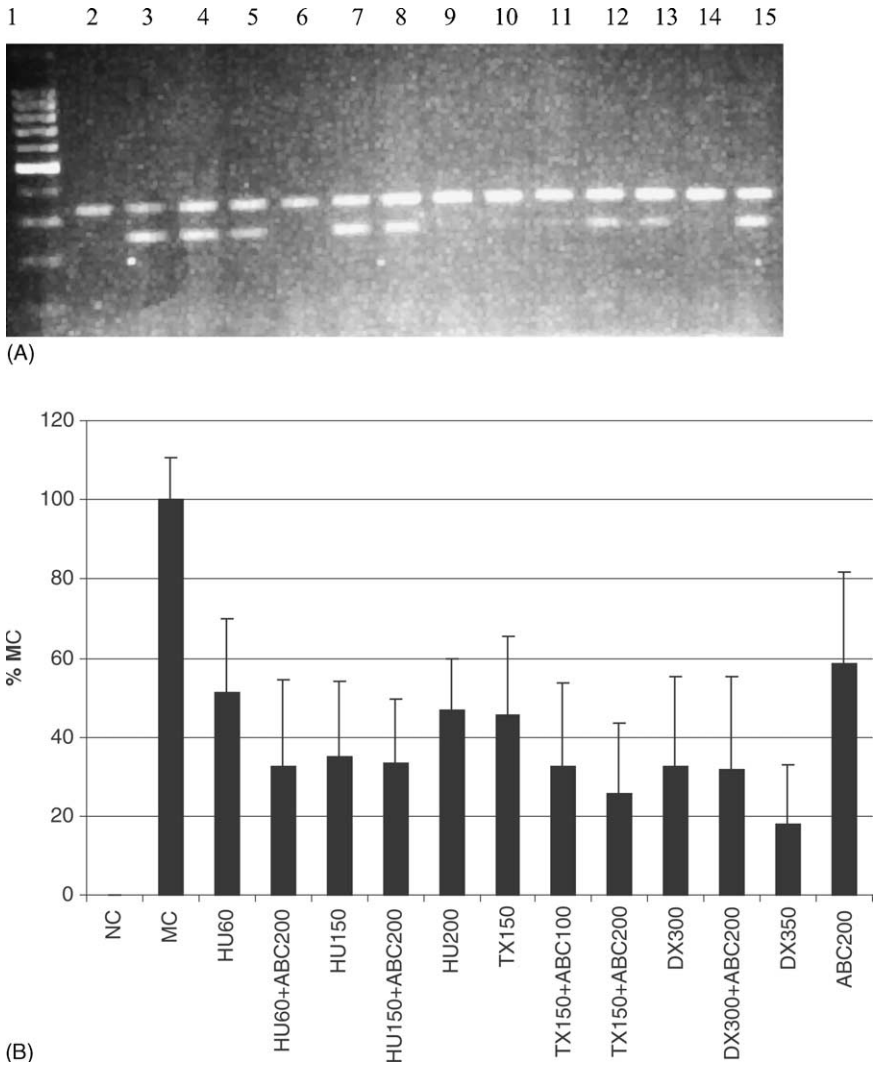

Fig. 4. (A) Effects of drug treatment on levels of splenic proviral DNA in LPBM5 MuLV-infected mice. The top row of bands represents β-actin, and the bottom row of bands represents BM5-def. Lane 1: molecular weight marker; lane 2: NC; lane 3: MC; lane 4: HU60; lane 5: HU60 + ABC200; lane 6: HU150 + ABC200; lane 7: HU200; lane 8: TX150; lane 9: TX150 + ABC100; lane 10: TX150 + ABC200; lane 11: DX300; lane 12: DX300 + ABC200; lane13: DX350; lane 14: DX350 + ABC200; lane 15: ABC200. The bands above show a representative example from each experimental group. (B) Effects of drug treatment on levels of splenic pro-viral DNA in LPBM5 MuLV-infected mice. Densitometry readings from semi-quantitative PCR are represented here as percentages of the infected control animals. Bars represent the mean and standard deviation of three mice per group.

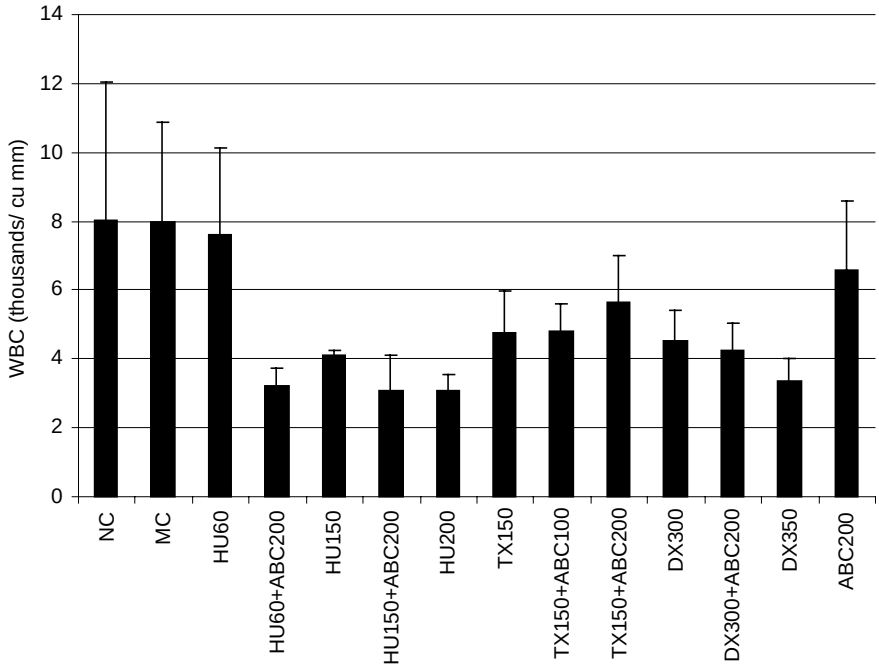

Fig. 5. Effects of drug treatment on WBC in LPBM5 MuLV-infected mice. WBC is expressed as thousands of cells per cubic millimeter. Bars represent the mean and standard deviation of at least three mice per group.

In the most effective anti-viral HU regimens (HU200; HU150 + ABC200), the red blood cell count (RBC) was significantly lower than the RBC from MC. Treatment with DX350 also significantly reduced the RBC values compared to MC, but no other regimen produced significant alterations in the RBC values.

The most effective anti-viral regimens containing HU (HU200; HU150 + ABC200) significantly lowered hematocrit (HCT) values compared to infected controls. No other therapeutic regimen significantly altered HCT values compared to infected controls.

HU200 and DX350 produced platelet count values that were significantly lower than non-infected control values, but they were not significantly lower than infected control values.

3.6. Effects of drug treatment on hematopoietic progenitor cells

Drug treatment regimens containing HU150, HU150 + ABC200, and HU200 produced significantly low numbers of femoral CFU-GM colonies compared to infected control values, which were significantly lower than non-infected control values. No other treatment groups lowered the femoral CFU-GM, and in fact, regimens containing DX actually increased the numbers of CFU-GM colonies compared to non-infected controls. This is also true of ABC monotherapy (Fig. 6).

No significant effects of femoral BFU-E colony numbers were observed in any drug treatment groups (data not shown).

4. Discussion

The cellular enzyme RR has been determined to be the rate-limiting step in de novo synthesis of dNTPs (Thelander and Reichard, 1979). Inhibition of RR by HU results in a reduction of dNTP pools, followed by a cessation in DNA synthesis and arrest of the cell cycle in S-phase (Moore and Hurlbert, 1985). HU has also been shown to cause cell death when used at high doses, or for prolonged exposures (Moore and Hurlbert, 1985). These properties of HU make it an interesting compound with potential benefits for HIV-infected patients. For example, the reduction of dNTP pools by HU may have an effect on HIV replication because HIV has been demonstrated to be extremely sensitive to fluctuations in dNTP levels (Gao et al., 1993; Meyerhans et al., 1994). The effects of HU upon dNTP levels also allows the possibility that HU could enhance the activity of nucleoside or -tide analog RT inhibitors because the nRTIs are structural analogs of the physiological dNTPs (Sommadossi, 1993), and compete with those physiological dNTPs for binding to the RT enzyme of HIV. It has been established that the activity of nRTIs is not completely dependent upon the actual level of nRTI, but lies mainly in the level of nRTI to its physiological counterpart (Johns and Gao, 1998). Therefore, reducing the endogenous level of dNTP may prove to be as effective as increasing the level of nRTI (Johns and Gao, 1998). At this point, HU has only been demonstrated to most consistently potentiate the effects of adenosine analogs (particularly ddI), with lesser potentiation of thymidine, cytosine, and guanosine analogs (Gao et al., 1994; Lori et al., 1994). In contrast to HU, TX and DX have been demonstrated to

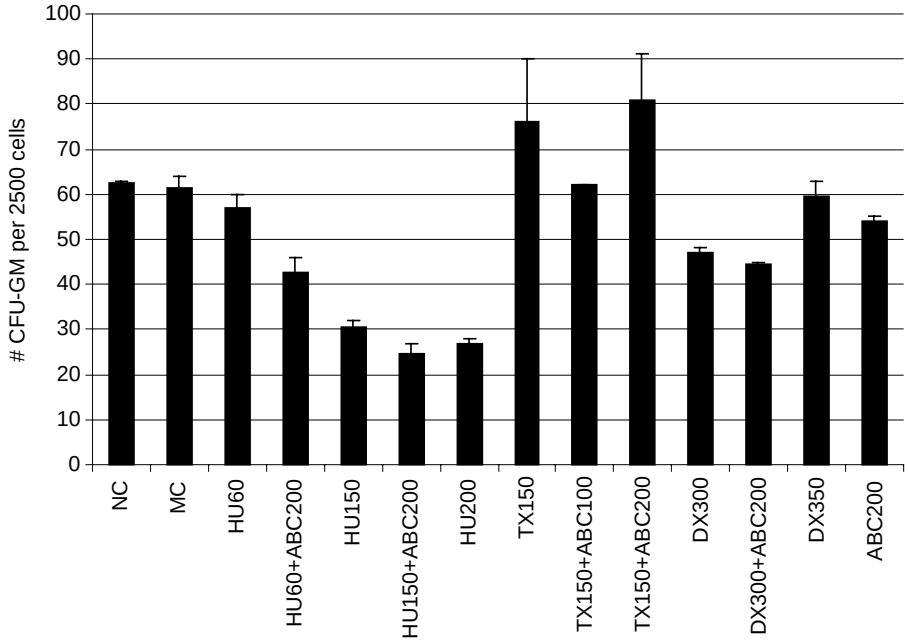

Fig. 6. Effects of drug treatment on bone marrow progenitor cells (CFU-GM) in LPBM5 MuLV-infected mice. The numbers of progenitor colonies (CFU-GM) are represented here as the number of colonies per 25,000 femur cells plated as described in the Section 2. Bars represent the mean and standard deviation of duplicate counts of pooled bone marrow from each group.

significantly reduce not only the dATP pool, but also the dCTP and dGTP pools in various cell lines (Elford and van't Riet, 1985; Szekeres et al., 1994a; Tihan et al., 1991). Based on this information our group speculated that TX and DX may have the ability to increase the anti-HIV-1 activity of a larger number of ddNs than HU, potentially providing more therapeutic options in the treatment of human AIDS.

The data presented here clearly indicate that the RRIs HU, TX, and DX are effective treatments with the ability to influence several parameters associated with disease progression in the murine AIDS model of retrovirus infection and are able to improve the activity of the guanine-like RT inhibitor ABC in the murine AIDS model without inducing significant hematological toxicity.

RRI doses used in this study were based on concentrations previously demonstrated by our laboratory to enhance the activity of ddI (Mayhew et al., 1997) and doses of ABC were chosen based upon recommendations by the manufacturer and upon the results of a 24 h maximum tolerated dose experiment (data not shown). Notable observations made in this study were that all RRIs provided effective inhibition of viral-induced disease pathophysiology, however, the use of HU while highly effective is associated with perturbations of the hematological system. This is in contrast to TX and DX, which were similarly effective, but induced far fewer perturbations to the hematological system than HU. This observation is confirmed by other studies from our laboratory (Mayhew et al., 1997, 1999, 2002b). Of particular interest was the observation that all RRIs could safely be combined with ABC, improving the activity of ABC in the model, as well as demonstrating that ABC improves the activity of sub-optimal doses of the RRIs. Although the improvement of RRI doses by the addition of ABC to the regimen was not significantly different from RRI monotherapy, general trends were clearly visible, however, statistical significance was difficult to obtain due to the high level of efficacy demonstrated by RRI monotherapy. Given the level of activity and the lack of significant toxicity observed in this study with TX and DX when combined with ABC, and the recent demonstration by Mayhew et al. (2002a) that TX and DX can reverse late-stage disease pathology, it would be of interest to administer these combination regimens to animals with late-stage infection.

With respect to the measurements of hematological toxicity, HU and DX were both associated with lowered WBC values compared to non-infected and infected controls, however, HU significantly depleted the number of committed hematological progenitor cells in the bone marrow compartment. This is in contrast to DX. DX actually increased the numbers of bone marrow progenitors. At the doses of TX used, no hematological perturbations were observed when TX was used alone or in combination with ABC.

LPBM5 MuLV viremia was not directly measured in this experiment, but it was indirectly measured by evaluation of levels of proviral DNA in spleens of infected animals treated with RRIs. All RRIs showed potent activity at reducing the levels of proviral DNA, with little difference in activity among groups. The main difference in treatment among groups is that the most effective regimens containing HU were associated with hematological toxicity as reflected in the lower peripheral blood and bone marrow indices.

In addition to these findings, previous experiments by our group in the HuPBMC SCID model of HIV-1 infection have demonstrated that TX and DX are able to reduce HIV-1 RNA titers in vivo (Broud et al., 1998; Ussery et al., 1999). Therefore, TX and DX may have a direct inhibitory effect upon HIV-1 replication if administered to infected individuals.

HIV-1 replication is characterized by T-cell activation, with the most efficient virus replication occurring in activated T cells (Stevenson et al., 1990). In HIV-1 infection, CD8+ cells increase dramatically (Giorgi et al., 1993), and CD8+ cells have been implicated in the immunopathology of HIV infection (Zinkernagel and Hengartner, 1994). RRIs TX and DX may have the same beneficial effects as HU upon CD4+ and CD8+ cell activation (Lori, 1999). In support of this idea is the observation that TX and DX inhibited virus induced B-lymphocyte activation and proliferation (characterized by hypergammaglobulinemia and splenomegaly) in the MAIDS model.

In conclusion, the ability of the RRIs HU, TX, and DX to improve the activity of ABC in the MAIDS model was comparatively examined. All three RRIs showed the ability to improve the activity of ABC in the MAIDS model, as determined by inhibition of splenomegaly, hypergammaglobulinemia, and proviral DNA levels, however, in the most effective regimens containing HU, disturbances in hematological function were observed. This is in stark contrast to TX and DX, which showed similar potency to HU containing regimens, but with little or no disturbance in hematological function at the doses examined. In light of these observations, it is possible that TX and DX may prove to be of benefit to HIV-1-infected individuals when combined with current treatment regimens.

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