PRELIMINARY COMMUNICATION

Neuroprotective and Anti–Human Immunodeficiency Virus Activity of Minocycline

M. Christine Zink, DVM, PhD
Jennifer Uhrlaub, BS
Jesse DeWitt, BS
Tauni Voelker, BS
Brandon Bullock, MS
Joseph Mankowski, DVM, PhD
Patrick Tarwater, PhD
Janice Clements, PhD
Sheila Barber, PhD

Context The prevalence of human immunodeficiency virus (HIV) central nervous system (CNS) disease has not decreased despite highly active antiretroviral therapy. Current antiretroviral drugs are expensive, have significant adverse effects including neurotoxicity, and few cross the blood-brain barrier.

Objective To examine the ability of minocycline, an antibiotic with potent anti-inflammatory and neuroprotective properties, to protect against encephalitis and neurodegeneration using a rapid, high viral load simian immunodeficiency virus (SIV) model of HIV-associated CNS disease that constitutes a rigorous in vivo test for potential therapeutics.

Design and Subjects Five SIV-infected pigtailed macaques were treated with 4 mg/kg per day of minocycline beginning at early asymptomatic infection (21 days after inoculation). Another 6 macaques were inoculated with SIV but remained untreated. Blood and cerebrospinal fluid (CSF) samples were taken on days 7, 10, 14, 21, 28, 35, 43, 56, 70, 77, and 84, and all macaques were humanely killed at 84 days after inoculation, a time that corresponds to late-stage infection in HIV-infected individuals.

Main Outcome Measures Blood and CSF samples were tested for viral load by real-time reverse transcription–polymerase chain reaction and levels of monocyte chemoattractant protein 1 were quantitated by enzyme-linked immunosorbent assay. The presence and severity of encephalitis was determined by microscopic examination of tissues. Central nervous system inflammation was further assessed by measuring infiltration and activation of macrophages, activation of p38 mitogen-activated protein kinase and expression of amyloid precursor protein by quantitative immunohistochemistry.

Results Minocycline-treated macaques had less severe encephalitis ($P=.02$), reduced CNS expression of neuroinflammatory markers (major histocompatibility complex class II, $P=.03$; macrophage marker CD68 , $P=.07$; T-cell intracytoplasmic antigen 1 , $P=.03$; CSF monocyte chemoattractant protein 1, $P=.001$), reduced activation of p38 mitogen-activated protein kinase ($P<.001$), less axonal degeneration (β-amyloid precursor protein, $P=.03$), and lower CNS virus replication (viral RNA, $P=.04$; viral antigen, $P=.04$). In in vitro analysis, minocycline suppression of HIV and SIV replication in cultured primary macrophages did not correlate with suppression of activation of p38-mitogen-activated protein kinase pathways, whereas suppression in primary lymphocytes correlated with suppression of p38 activation.

Conclusions In this experimental SIV model of HIV CNS disease, minocycline reduced the severity of encephalitis, suppressed viral load in the brain, and decreased the expression of CNS inflammatory markers. In vitro, minocycline inhibited SIV and HIV replication. These findings suggest that minocycline, a safe, inexpensive, and readily available antibiotic should be investigated as an anti-HIV therapeutic.

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HUMAN IMMUNODEFICIENCY VIRUS (HIV) central nervous system (CNS) disease is characterized by infiltration and activation of macrophages and microglia, production of proinflammatory cytokines, expression of proapoptotic and neurotoxic mediators, and neuronal loss.[1-3] Although many antiretroviral drugs suppress HIV replication in peripheral blood, few drugs achieve effective levels in the brain or alter the inflammatory responses that accompany viral infection in the CNS. In addition, many reverse transcriptase and protease inhibitors are expensive and have significant adverse effects, including neurotoxicity.[4,5] A number of neuroprotective agents for HIV-infected individuals are being examined in clinical trials,[6-9] but no single agent has emerged as a solution to both the inflammatory and neurodegenerative effects of HIV in the CNS.

The simian immunodeficiency virus (SIV)–macaque model provides an excellent system to dissect the patho-

Author Affiliations are listed at the end of this article.
Corresponding Author: M. Christine Zink, DVM, PhD, Johns Hopkins University School of Medicine, 733 N Broadway, Room 839, Baltimore, MD 21205 (mczink@jhmi.edu).

genesis of HIV-induced CNS disease because it recapitulates key features of HIV CNS infection, including the development of encephalitis with active virus replication in the CNS, characteristic histopathological changes, psychomotor impairment, and neurodegeneration.[10-13] However, the prolonged course of infection (years) and the unpredictable incidence of SIV CNS disease in the classic SIV-macaque model have limited its usefulness to elucidate pathogenic mechanisms of HIV CNS disease that may be vulnerable to therapeutic intervention. We therefore developed an accelerated, consistent SIV-macaque model of HIV CNS disease in which more than 90% of infected animals develop encephalitis with neurodegeneration 3 months after inoculation.[13,14] Because of the high viral loads in plasma and CSF of infected animals, the rapidity of immunosuppression and the high incidence of severe neurological disease, this model provides a rigorous test for evaluation of anti-HIV and neuroprotective therapeutics.

We tested the ability of the tetracycline derivative minocycline to protect against SIV neurodegeneration for several reasons. First, minocycline has potent anti-inflammatory properties and effectively crosses the blood-brain barrier.[15-17] Second, minocycline is neuroprotective in animal models of amyotrophic lateral sclerosis, multiple sclerosis, Parkinson disease, Huntington disease, and ischemic or traumatic brain injury and has recently been shown to reduce gadolinium-enhancing lesions in the CNS of patients with multiple sclerosis.[18-24] Third, the anti-inflammatory and neuroprotective properties of minocycline in several animal models have been linked to suppressed activation of p38 mitogen-activated protein kinase, and we recently demonstrated increased p38 activation in SIV encephalitis.[25] Finally, minocycline is readily available, inexpensive, relatively safe when administered long term, and it is approved by the US Food and Drug Administration for treatment of other medical conditions.

METHODS

Viruses and Animal Studies

Twelve juvenile pigtailed macaques (*Macaca nemestrina*) were intravenously inoculated as previously described with SIV/DeltaB670 (50 AID_{50}) and SIV/17E-Fr (10 000 AID_{50}).[12] Minocycline at a dose of 4 mg/kg per day divided over 2 doses was administered orally in a cherry-flavored tablet to 6 macaques starting 21 days after innoculation.[26] One macaque in the treated group who frequently refused to eat the minocycline tablet was removed from the study. There were no recognizable adverse effects of minocycline treatment. Cerebrospinal fluid and plasma samples were taken on days 7, 10, 14, 21, 28, 35, 43, 56, 70, 77, and 84 for quantitation of viral RNA and monocyte chemoattractant protein 1 (MCP-1).[12,27] All macaques were killed 84 days after inoculation in accordance with federal guidelines and institutional policies. At death, macaques were perfused with sterile saline to remove blood from the vasculature prior to freezing or fixing tissues. These animal studies were approved by the Johns Hopkins Animal Care and Use Committee. All the animals were humanly treated in accordance with federal guidelines and institutional policies.

Laboratory Assessment

Pathological Assessment. All tissues were examined microscopically by 2 pathologists (M.C.Z., J.M.). Sections of frontal and parietal cortex, basal ganglia, thalamus, midbrain, cerebellum, brain stem, and spinal cord were examined microscopically and scored independently as mild, moderate, or severe according to previously described criteria.[12]

Quantitation of RNA in Plasma, CSF, and Brain. Viral RNA in plasma and CSF and viral RNA in brain tissue (basal ganglia) were quantitated by real-time reverse transcription–polymerase chain reaction using a protocol previously described.[12]

Quantitation of MCP-1 in Plasma, CSF, and Brain. Monocyte chemoattractant protein 1 levels in CSF, plasma, and brain homogenates were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minn) as described previously.[27]

Quantitative Immunohistochemical Analysis. Our methods for quantitative immunohistochemical analysis of macrophage marker CD68, major histocompatibility complex class II, T-cell intracytoplasmic antigen 1, SIV glycoprotein 41, β-amyloid precursor protein, and activated p38 have been described previously.[12,25,27,28]

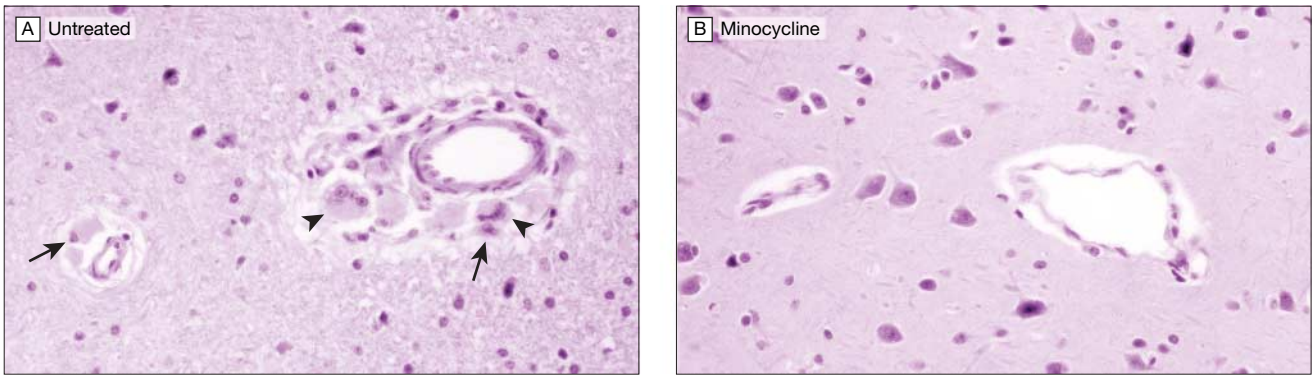
Cell Culture, In Vitro Infection, and Treatment With Minocycline. Peripheral blood lymphocytes and macrophages were cultured as described.[29] Minocycline was added to cultures 24 hours before inoculation with HIV-1IIIB, HIV-1Ba-L, SIV/17E-Fr, or SIV/DeltaB670. Peripheral blood lymphocytes and macrophages were infected (multiplicity of infection, 0.005 and 0.05, respectively), washed, and cultured with or without minocycline. Virus replication was assessed by quantitating p27 or p24 by enzyme-linked immunosorbent assay (Coulter, Miami, Fla) in supernatants collected periodically after infection. Viabilities of infected peripheral blood lymphocytes (assessed by trypan blue exclusion) were not affected by minocycline. Minocycline concentrations of 20 µg/mL or less were not toxic to macrophages; however, concentrations of 40 µg/mL resulted in an approximate 30% reduction in viability after 9 days.

Western Blot Analysis of p38 Activation. For HIV- and SIV-infected cultures, whole cell lysates were prepared from the above cultures 9 days after inoculation and were subjected to Western blot analysis for total p38 or activated p38 (Cell Signaling, Beverly, Mass).

Statistical Analysis

Fisher exact *P* value was used to compare the proportions of animals with moderate or severe encephalitis between treated and control groups to properly account for the small number of observations. The dichotomization of CNS severity (moderate to se-

Figure 1. Brain Sections of Macaques Infected With Simian Immunodeficiency Virus



A, Brain section from an SIV-infected untreated macaque demonstrating typical changes of severe SIV encephalitis with numerous epithelioid macrophages (arrows) and multinucleated giant cells (arrowheads) in perivascular spaces. B, Brain section from an SIV-infected macaque treated with minocycline, showing no inflammation. Hematoxylin-eosin; original magnification ×200.

vere vs none to mild) has been used in previous research using this model[27] and was conducted prior to data analysis. The comparison between treated and control groups' mean MCP-1 level in the brain was conducted using a 2-sample *t* test. Expression of major histocompatibility complex class II, CD68, T-cell intracytoplasmic antigen 1, p-p38, β-amyloid precursor protein, and viral protein in the brain were quantitated using 20 repeated measures on each tissue sample. The *P* values were determined by comparing the replicate data from each group of animals with the data from the other group although only the means were depicted graphically in the figures. Comparisons between the 2 treatment groups were conducted using a linear regression model with a dichotomous variable indicating membership in the treatment group, a method analogous to the 2-sample *t* test. In addition, the coefficient and SE estimates were calculated using generalized estimating equations,[30] a robust estimation procedure that properly accounts for the inherent correlation among replicated or repeated measurements observed on the same animal. For variables with measurements taken repeatedly overtime (ie, CSF viral RNA and CSF:plasma MCP-1 ratio) we again used generalized estimating equations in a linear regression model but with time from inoculation and treatment by time interaction included as independent variables. The model estimates of the coefficient and standard error for the interaction term were used to test the differences, if any, of the rates of change over time between the 2 treatment groups. All statistical tests were performed as 2-sided tests. Analyses were performed using Stata statistical software version 8 (StataCorp, College Station, Tex). Statistical significance was $P<.05$.

RESULTS

CNS Inflammation and Axonal Degeneration

In this study, a rapid, high viral load SIV model of HIV CNS disease was used to test the ability of minocycline to suppress SIV CNS inflammation and neurodegeneration. Because all macaques develop rapid immunosuppression and express high viral loads in plasma and CSF and because the majority develops encephalitis within 84 days after virus inoculation, this model provides a rigorous test for potential therapeutic treatments. Five macaques were infected with SIV[12] and received minocycline treatment (4 mg/kg per day)[26] that was initiated during asymptomatic infection (21 days after inoculation).[31] By day 84 after inoculation, of the untreated macaques, 3 developed moderate, 2 developed severe, and 1 developed no SIV encephalitis (FIGURE 1).[12] In contrast, 3 of 5 minocycline-treated animals did not develop encephalitis while the remaining 2 macaques had only mild encephalitis. This decreased incidence of moderate and severe encephalitis in the minocycline-treated macaques was statistically significant ($P=.02$).

To assess the ability of minocycline to suppress SIV-induced CNS inflammation, we quantitated infiltration and activation of macrophages and infiltration of cytotoxic lymphocytes into the CNS, activation of p38 mitogen-activated protein kinase in the brain and expression of β-amyloid precursor protein, a marker of axonal degeneration, by quantitative immunohistochemical analysis.[12,25,27,28] Minocycline significantly reduced expression of major histocompatibility complex class II antigens ($P=.03$; FIGURE 2A), indicating suppressed activation of macrophages, endothelial cells, or both in the brain.

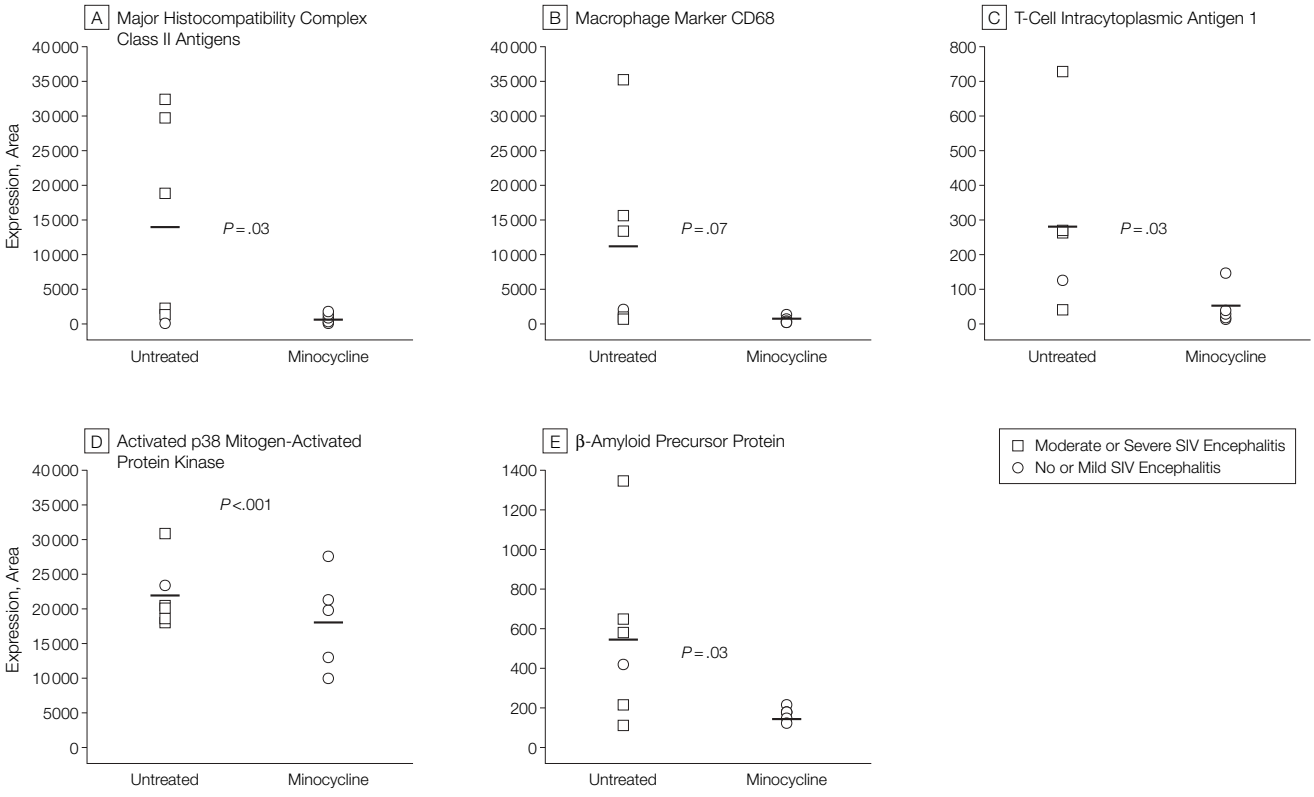
Decreased infiltration and activation of macrophages in minocycline-treated animals also was suggested by reduced expression of the macrophage marker CD68 ($P=.07$, Figure 2B). Minocycline also reduced the infiltration of cytotoxic lymphocytes into the brain as evidenced by significantly reduced expression of the cytotoxic lymphocyte marker T-cell intracytoplasmic antigen 1 ($P=.03$,

Figure 2C) in treated animals. The effects of minocycline were not only limited to macrophages and lymphocytes as suggested by the finding of significantly reduced activation of p38 in neurons and astrocytes in the brain of minocycline-treated macaques ($P<.001$, Figure 2D). Expression of β-amyloid precursor protein, an established marker of axonal degeneration, also was significantly lower ($P=.03$, Figure 2E) in minocycline-treated macaques compared with untreated SIV-infected animals.

MCP-1 Expression in the CNS

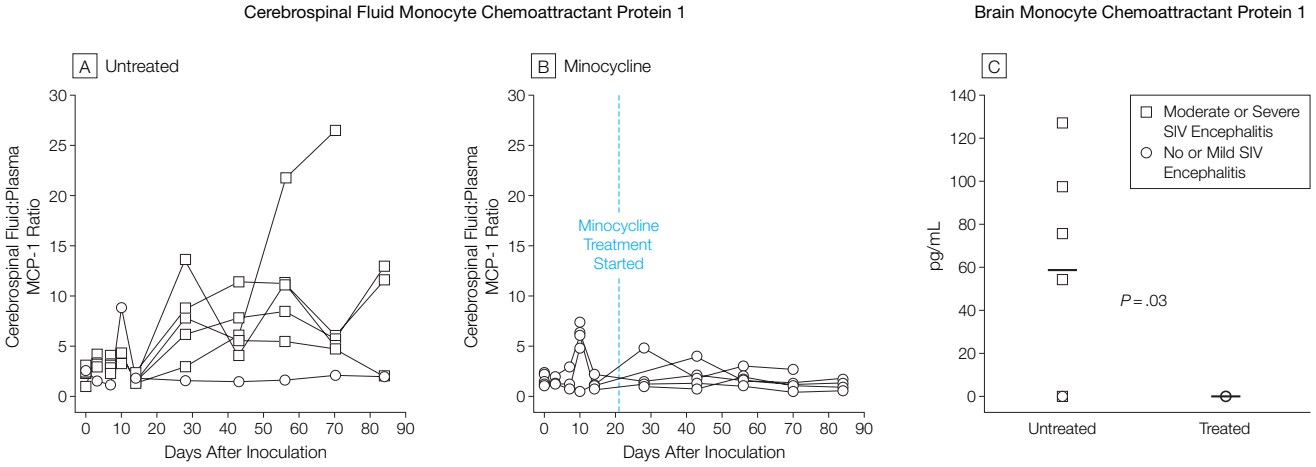
Proinflammatory chemokines, particularly MCP-1, provide signals from the CNS that promote infiltration of macrophages and to a lesser extent inflammatory T lymphocytes from the periphery into the brain. Increased CSF expression of MCP-1 has been correlated with the development of HIV and SIV CNS disease.[27,32,33] Quantitation of CSF:plasma MCP-1 levels provides a measure of the gradient of the MCP-1 that exists between the CNS and the periphery.[27] To determine whether minocycline affects expression of this important proinflammatory signal in the CNS, MCP-1 levels in CSF and plasma were quantitated throughout infection and MCP-1 in brain was quantitated at necropsy. Cerebrospinal fluid MCP-1 levels (as measured by CSF:plasma ratios) of several treated and untreated SIV-infected macaques increased during acute infection and then declined after 10 to 14 days after inoculation (FIGURE 3A, B), a pattern typical of acute SIV infection.[27] In the untreated SIV-infected macaques, CSF MCP-1 levels increased again after 28 days in contrast to the minocycline-treated macaques in which CSF MCP-1 levels remained significantly lower ($P=.001$). The low levels of MCP-1 in the CSF of the minocycline-treated macaques during late-stage infection were confirmed by measuring MCP-1 protein levels in brain homogenates by enzyme-linked immunosorbent assay. Minocycline-treated macaques had significantly lower levels of MCP-1 protein in the brain than SIV-infected macaques ($P=.03$;

Figure 2. Suppression of Simian Immunodeficiency Virus–Induced Central Nervous System Inflammation and Axonal Degeneration by Minocycline



A, Expression of major histocompatibility complex class II antigens, which detects activated macrophages and microglia and endothelial cells. B, Expression of macrophage marker CD68, which marks activated macrophages and microglia. C, Expression of T-cell intracytoplasmic antigen 1, a protein found in cytotoxic cytoplasmic granules of natural killer cells and CD8 T cells. D, Expression of activated p38 mitogen-activated protein kinase (p-p38); up-regulated most in neurons and astrocytes in our model. E, Expression of β-amyloid precursor protein, a marker of axonal degeneration. Each data point represents the mean of 20 repeated measures of major histocompatibility complex class II, CD68, T-cell intracytoplasmic antigen 1, p-p38, or β-amyloid precursor protein. The *P* values were determined by comparing the replicate data from each group of animals with the data from the other group. For graphical presentation, only the animal-specific means are depicted. Horizontal bars indicate group means.

Figure 3. Expression of Monocyte Chemoattractant Protein 1 in the Central Nervous System



Monocyte chemoattractant protein 1 (MCP-1) expression in cerebrospinal fluid (CSF) is expressed as the ratio of MCP-1 in the CSF vs that in the plasma. A, After 28 days after inoculation, untreated macaques with moderate to severe encephalitis had increased CSF:plasma MCP-1 ratios. B, Minocycline-treated macaques had significantly lower CSF:plasma MCP-1 ratios ($P = .001$) after 28 days. C, Simian immunodeficiency virus–infected, minocycline-treated macaques had significantly lower MCP-1 protein in brain homogenates than untreated macaques. Horizontal bars indicate group means.

Figure 3C). Thus, minocycline has an impact on the release of chemokines that mediate influx of inflammatory cells into the brain.

SIV Replication in the CNS

Because previous studies in this model demonstrated that macaques with more severe encephalitis had higher levels of viral RNA in the CSF and brain, we quantitated viral RNA in the CSF and in brain tissue from SIV-infected minocycline-treated and untreated macaques by real-time reverse transcription–polymerase chain reaction.[12] Minocycline significantly suppressed levels of viral RNA in the CSF after 28 days. ($P = .05$; FIGURE 4A, B). Comparison of the change in CSF viral load from day 28 after inoculation onward using a linear regression model demonstrated declining CSF viral loads in minocycline-treated macaques (dark line after 28 days in Figure 4A, B) in contrast to increasing CSF viral loads in untreated macaques. Furthermore, minocycline-treated macaques had reduced expression of viral RNA in the basal ganglia region of the brain ($P = .04$, Figure 4C). The suppressive effect of minocycline on SIV replication was confirmed by quantitative image analysis on brain sections stained immunohistochemically for SIV glycoprotein 41. Minocycline-treated macaques had significantly lower expression of viral antigen in brain than untreated SIV-infected macaques ($P = .04$; Figure 4D).

HIV and SIV Replication in Primary Lymphocytes and Macrophages

Since minocycline suppressed SIV replication in the CNS, we examined whether it also would inhibit SIV and HIV replication in the predominant target cells productively infected by these viruses—macrophages and lymphocytes. Cultured primary macaque and human peripheral blood monocular cells–derived macrophages and lymphocytes[29] were treated with 10, 20, or 40 µg/mL of minocycline[20,34] for 24 hours prior to inoculation with the appropriate viruses. Virus replication was quantitated over time by p24 (HIV) or p27 (SIV) enzyme-linked immunosorbent assay on culture supernatants. Minocycline inhibited HIV and SIV replication in primary lymphocytes (FIGURE 5A, B, E) and macrophages (Figure 5C, D, and F), in a dose-dependent manner. At the highest dose, minocycline inhibited HIV replication by 92% and 99% and SIV replication by 99% and 85% in lymphocytes and macrophages, respectively.

To investigate the mechanism(s) involved in minocycline-mediated suppression of HIV and SIV replication, we examined activation of p38 in cultured, HIV-infected minocycline-treated primary lymphocytes and macrophages. p38 is ubiquitously expressed and although activation of p38 in astrocytes and neurons accompanies neurodegeneration in our model and others, this kinase also has critical signaling functions in other cells including T lymphocytes and monocytes or macrophages.[16,25,34] We specifically examined p38 activation as a potential mechanism for virus suppression because 2 reports have suggested that activation of p38 is required for replication of HIV in lymphocytes[35] and in U1 promonocytic cells[36] and because minocycline suppresses activation of p38 in response to proinflammatory cytokines and neurotoxic products.[16,34] Cells were treated with minocycline for 24 hours prior to HIV infection and activation of p38 was assessed by Western blot analysis of whole cell lysates prepared days 9 after inoculation. Minocycline inhibited activation of p38 (and to some extent constitutive ex-

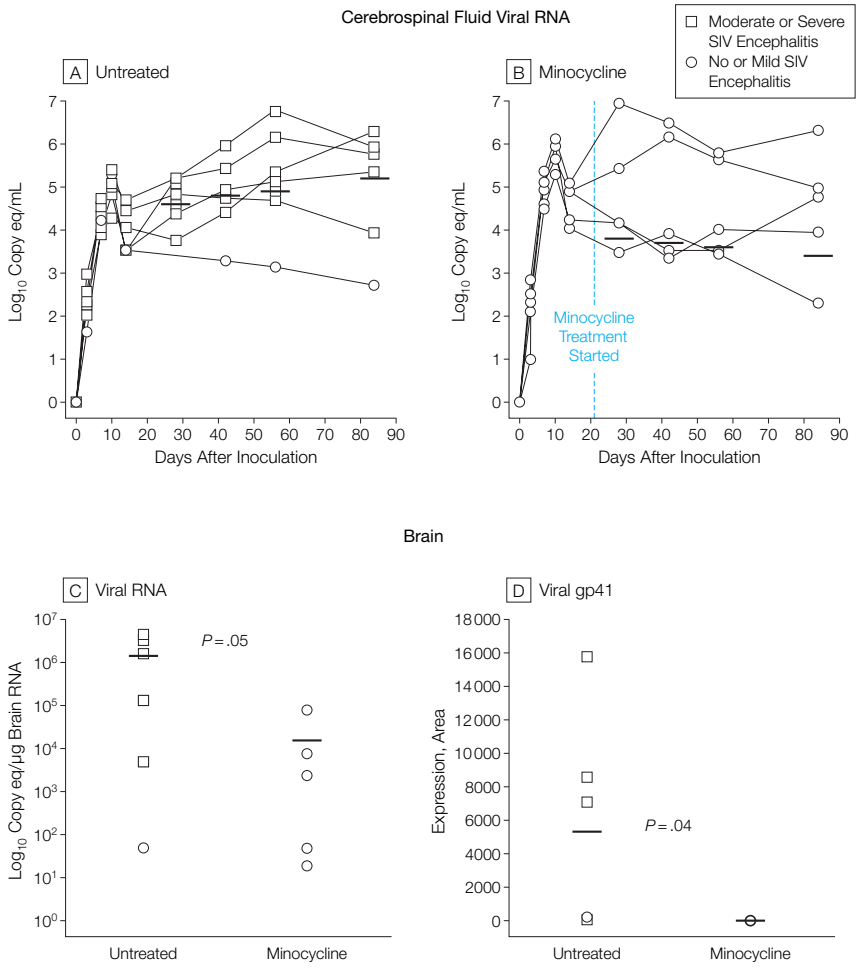
pression of p38) in HIV-infected primary lymphocytes but not in macrophages (FIGURE 6A, B). Similar results were obtained from SIV-infected primary lymphocytes and macrophages (data not shown). These data indicate that although inhibition of virus replication in minocycline-treated primary lymphocytes correlates with inhibition of p38 activation, inhibition of HIV and SIV replication in primary macrophages occurs via p38-independent mechanisms. They further indicate that the known activators of p38 in both lymphocytes and macrophages, mitogen-activated protein kinase kinase 3 and 6, are not direct targets of minocycline.

COMMENT

In this study, we demonstrate that minocycline significantly inhibits HIV and SIV replication in vitro and also reduces the incidence and severity of encephalitis in a rigorous SIV-macaque model of HIV CNS disease. The latter observation is particularly impressive, given the rapidity and severity of SIV encephalitis in our model and the ability of minocycline to intervene effectively when treatment is initiated during asymptomatic infection and continued during the short period between 21 and 84 days. To the best of our knowledge, this is the first report demonstrating anti-inflammatory and neuroprotective activity of an antibiotic against a highly pathogenic virus infection and that minocycline suppresses HIV and SIV replication in lymphocytes and macrophages, the main target cells in vivo for these viruses. Given that the prevalence of HIV CNS disease has not declined in the era of highly active antiretroviral treatment, this finding may have important implications for future studies on the prevention and treatment of HIV-infected individuals.[37-39]

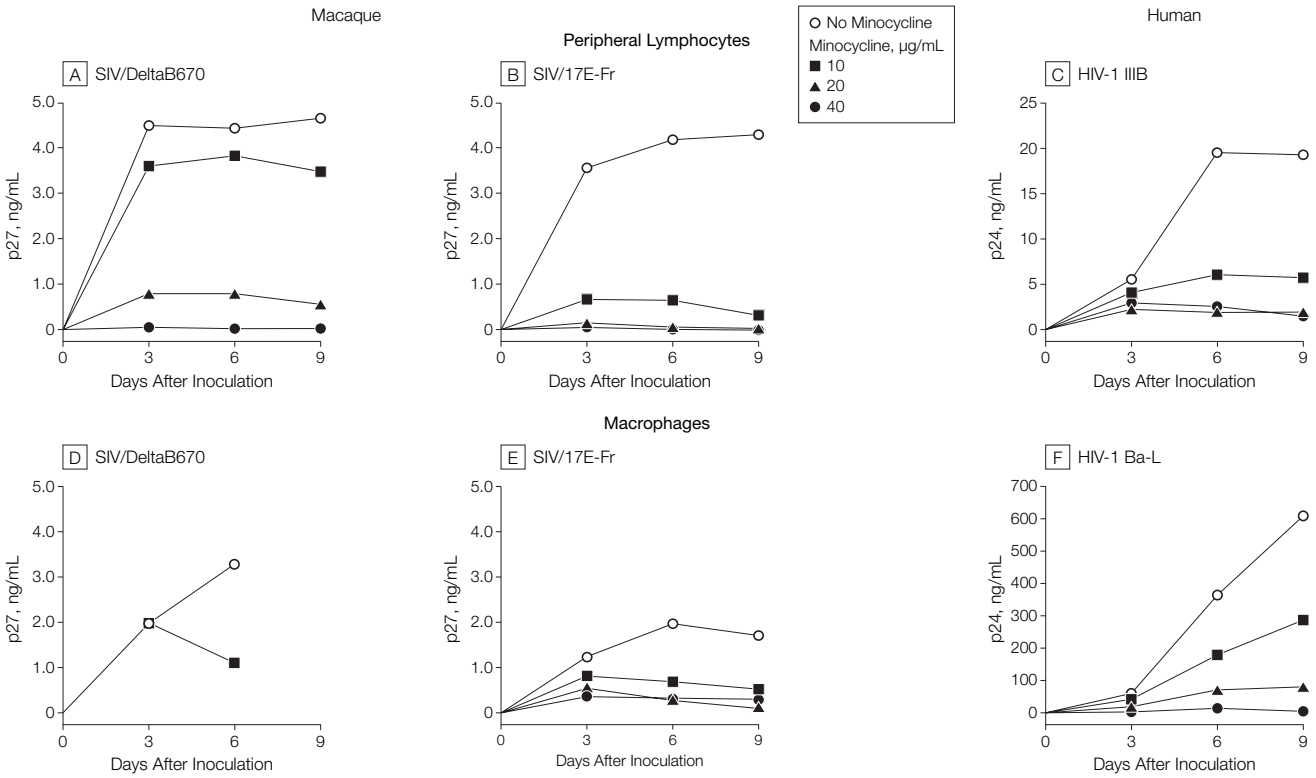
The ability of minocycline to prevent increased expression of MCP-1 in the brain is unquestionably one factor mediating the neuroprotective effect in our SIV model of HIV CNS disease.[27] Monocyte chemoattractant protein 1, which is produced by macrophages and astrocytes in the brain, is the major inflammatory chemokine responsible for the influx of macrophages into the brain. Macrophages provide a primary mode of transport for HIV and SIV into the brain, are the major sources for HIV and SIV replication in the CNS and produce toxic mediators during HIV CNS disease. Our finding of reduced activation of macrophages or microglia and decreased influx of cytotoxic lymphocytes in minocycline-treated SIV-infected macaques is consistent with the suppression of MCP-1 expression in the CNS by this antibiotic. This study is the first, to our knowledge, to link MCP-1 to the mechanisms mediating the neuroprotective effects of minocycline. This finding suggests that minocycline may have clinical applicability to neurodegenerative disorders in which MCP-1-induced infiltration and activation of macrophages is an important determinant of neuropa-

Figure 4. Suppression of Viral Gene Expression in the Central Nervous System by Minocycline



Cerebrospinal fluid (CSF) viral RNA quantitated by real-time reverse transcription–polymerase chain reaction in the simian immunodeficiency virus (SIV)–infected, untreated macaques, A, was significantly higher from 28 to 84 days after inoculation ($P = .05$) than in SIV-infected, minocycline-treated macaques during the same period, B. The area to the right of the vertical dashed line indicates samples taken after the initiation of minocycline treatment. C, Minocycline-treated animals had significantly less viral RNA in brain homogenates. D, Minocycline-treated animals also had significantly less viral glycoprotein 41 protein in the brain. For C and D, each data point represents the mean of 20 repeated measures of glycoprotein 41. The *P* value was determined by comparing the replicate data from each group of animals with the data from the other group. For graphical presentation, only the animal-specific means are depicted. Horizontal bars indicate group means.

Figure 5. Human Immunodeficiency Virus and Simian Immunodeficiency Virus Replication in Primary Lymphocytes and Macrophages

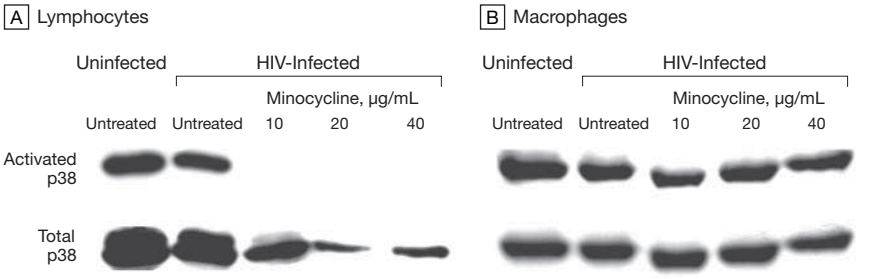


A and B, Simian immunodeficiency virus (SIV)–infected primary macaque lymphocytes, C, Human immunodeficiency virus (HIV)–infected primary human lymphocytes, D and E, SIV-infected primary macaque macrophages, and F, HIV-infected primary human macrophages.

thology, such as multiple sclerosis. A recent report suggested that minocycline may be an effective therapy for multiple sclerosis.[24] Furthermore, it is possible that minocycline treatment may have a role in treatment of HIV-infected individuals who are at higher risk for development of HIV CNS disease by virtue of a genetic polymorphism in the MCP-1 promoter region that increases MCP-1 levels.[33]

The dose of minocycline that was used in these macaques (4 mg/kg per day) is within in the tolerated range for humans. Although it can be difficult to compare effective or toxic doses from one species to another, studies in nonhuman primates come as close to actual human trials as possible. Two double-blind, randomized, placebo-controlled feasibility trials of minocycline in patients with amyotrophic lateral sclerosis have been reported recently.[40] In the first, 19 patients were treated with 200 mg/d of minocycline (3 mg/kg per day for a 70-kg individual) for 6 months with no difference in adverse events compared with those in the placebo group. In a second, 23 patients received up to 400 mg per day in an 8-month crossover trial. The mean tolerated dose in this study was 387 mg/d (5.5 mg/kg per day for a 70 kg individual). These findings suggest that minocycline at the dose that suppressed CNS inflammation and virus replication in macaques may be well tolerated in HIV-infected individuals.

Figure 6. Western Blot Analysis



Western blot analysis of total p38 and activated p38 (p-p38) in minocycline-treated, human immunodeficiency virus [HIV]–infected primary human lymphocytes, A, and macrophages, B, demonstrating minocycline-induced suppression of activation of p38 in lymphocytes but not macrophages.

Although minocycline treatment initiated at 21 days after SIV inoculation

significantly reversed the pattern of increasing levels of viral RNA in the CSF as infection progressed, its effect on plasma viral load was less marked, likely because of the early high plasma viral loads ($\approx 10^8$ copy Eq/mL by 10 days after inoculation) in this accelerated SIV-macaque model. Nevertheless, plasma viral RNA levels were significantly lower in the 3 minocycline-treated macaques that did not develop encephalitis than in the 2 treated macaques with encephalitis (data not shown; $P = .05$).

Perhaps the most unexpected result of these studies was the ability of minocycline to substantially inhibit replication of SIV and HIV in primary cultures of macrophages and lymphocytes. A recent study describing minocycline inhibition of HIV in cultured microglia was recently reported.[41] It seems unlikely that minocycline has classic antiviral activity as do reverse transcriptase and protease inhibitors because the antibiotic was not engineered to target a specific viral protein.

We propose that rather than exerting direct antiviral activity, minocycline modifies the intracellular or extracellular environment making it nonpermissive for HIV or SIV replication. The ability of minocycline to modify environments differentially in primary macrophages and T lymphocytes (as evidenced by the differential effect of minocycline on p38 activation) raises the possibility that each cell type has a unique minocycline-sensitive target and hence, a unique mechanism of suppression.[35,36,42] An important potential therapeutic advantage of this differential effect is that if the virus develops mutations that confer resistance to minocycline in one target cell type, that resistance might not confer a replicative advantage in the other cell type. Minocycline represents a second immunodulatory agent to suppress HIV replication in macrophages in a p38-independent manner. Murabutide, which is currently being studied in clinical trials of HIV-infected patients, also acts to suppress HIV replication in a p38-independent manner in macrophages.[42,43]

Minocycline is a semisynthetic second-generation tetracycline that readily crosses the blood-brain barrier, is inexpensive and safe, has been prescribed for years, and is available in generic form. Our findings that minocycline inhibits SIV and HIV replication in primary macrophages and lymphocytes in vitro and suppresses SIV replication in the brain and the accompanying neuropathology provide evidence for designing human studies to examine the potential role of minocycline as a supplement to highly active antiretroviral therapy in the treatment of HIV-associated cognitive disorders and in maintaining low viral loads in patients for whom highly active antiretroviral therapy must be discontinued. Furthermore, minocycline is efficacious against a variety of infectious diseases, including toxoplasmosis, malaria, and several sexually transmitted diseases and thus has potential for long-term use in global areas in which individuals frequently harbor multiple infections besides HIV.

Author Affiliations: Department of Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, Md (Drs Zink, Mankowski, Clements, and Barber; Mss Uhrlaub, and Voelker; and Messrs DeWitt and Bullock); Department of Biostatistics and Epidemiology, University of Texas Health Science Center at Houston, School of Public Health, El Paso Regional Campus, El Paso (Dr Tarwater).

Author Contributions: Dr Zink had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Zink, Tarwater, Clements, Barber.
Acquisition of data: Zink, Uhrlaub, DeWitt, Voelker, Bullock, Mankowski, Tarwater, Barber.
Analysis and interpretation of data: Zink, Mankowski, Tarwater, Clements, Barber.
Drafting of the manuscript: Zink, Uhrlaub, DeWitt, Voelker, Bullock, Mankowski, Tarwater, Clements, Barber.
Critical revision of the manuscript for important intellectual content: Zink, Clements, Barber.
Statistical analysis: Tarwater.
Obtained funding: Zink, Clements.
Administrative, technical, or material support: Zink, Uhrlaub, DeWitt, Voelker, Bullock, Mankowski, Clements, Barber.
Study supervision: Zink, Barber.
Financial Disclosures: Drs Zink and Barber are named as inventors on a patent pending for minocycline to treat HIV infection. The patent will be held by the Johns Hopkins University. Otherwise no other authors reported financial disclosures.
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Let no one be discouraged by the belief there is nothing one person can do against the enormous array of the world's ills, misery, ignorance, and violence. Few will have the greatness to bend history, but each of us can work to change a small portion of events. And in the total of all those acts will be written the history of a generation.

—Robert F. Kennedy (1925-1968)