2013

Nucleoside Reverse Transcriptase Inhibitors and Ototoxicity: A Review of the Literature

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Spring 2013

Chapter 1

Introduction

In December 2011, the World Health Organization released a report estimating the number of worldwide HIV/AIDS cases at 30 million. [1] HIV/AIDS is an antiretroviral disease that affects the immune system by destroying cells vital to the body's immune response. This is accomplished by two general mechanisms: killing the host cells, and killing uninfected neighboring cells. Both mechanisms are based on manipulation of the genetic information contained within ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) of a type of immune cell called the $CD4^+$ cell. [2]

In the early stages of HIV infection, the virus takes over the RNA replication mechanism of $CD4^+$ cells and begins to replicate. When infected RNA is used as a blueprint to manufacture DNA, the HIV virus's genetic information is inserted into the cell's DNA. This infected DNA is then used as a blueprint for more HIV viruses, and molecules that induce cell death, or apoptosis, called cytokines. The infected cell dies, releasing the HIV and cytokines into the blood stream. While the HIV virus attaches to neighboring cells, the cytokines attach to receptors on uninfected cells and induce apoptosis. As the virus continues to replicate, the $CD4^+$ cells die. When a person's $CD4^+$ cell count drops from the normal level of $1000/mm^3$ to below $200/mm^3$ of blood, the patient has progressed to the AIDS stage of the disease. After the disease progresses to AIDS, the patient's immune system is severely compromised. [2] Most people are aware of the disease's effects on the immune system, but few realize the impact that HIV/AIDS has on the auditory system.

In 1998, Moazzez and Alvi reported that anywhere from 20-50% of HIV/AIDS patients experience some degree of sensorineural hearing loss. These hearing losses can result from viral

attacks on the auditory nerve, as a byproduct of complications from opportunistic infections that attack the central nervous system, or as a side effect of ototoxic drugs, which affect the cochlea and/or auditory nervous system. [4] Instances of hearing loss in HIV patients are well documented. Marra et al. found that 29% of the patients in their 1997 study on the prevalence of hearing loss in HIV patients taking antiretroviral drugs had hearing loss, defined as a hearing threshold >25dBHL at 4000Hz. [5] Another study performed by McNaughten, Wan, and Dworkin in 2001 determined the prevalence of hearing loss among HIV-infected patients by examining data from the Adult Spectrum of HIV Disease (ASD) project. Of the 3646 patients enrolled in the ASD, 30 were identified as having a hearing loss with unspecified thresholds. This low prevalence was attributed to the practice of retroactively examining medical records. The investigators believe that the prevalence of hearing loss would increase if there were audiometry results on file for each patient. [6]

HIV patients experience both conductive and sensorineural hearing losses. Most conductive losses are caused by opportunistic infections. Some of the most common ear pathologies in HIV patients include otitis media, nasopharyngeal polyps, mastoiditis, and otitis externa associated with Kaposi's sarcoma or herpes zoster. [7] When Chao et al. conducted a study on the prevalence of hearing loss in HIV-positive children in Peru in 2011, they found that 34.5% of the subjects had a conductive hearing loss. Ninety-eight percent of these children were receiving highly active antiretroviral therapy (HAART), and a large number of the children presented with middle ear pathologies, including otitis media and abnormal tympanograms. The prevalence of hearing impairment in the HIV-positive children was five times higher than the prevalence of hearing impairment in HIV-negative children. [8]

While it is fairly simple to identify the cause of a conductive hearing loss, identifying sensorineural hearing losses and their causes is much harder. In 2008, Ranjan and Bhat compared the distortion product ototacoustic emissions (DPOAE) of HIV-positive individuals to those of HIV-negative individuals. Fifty percent of the HIV-positive patients in the experimental group demonstrated DPOAE readings that indicated subclinical sensorineural hearing loss. Because these losses occurred at frequencies higher than 1000Hz, it is possible that the affected patients had not yet noticed them. In this study, the researchers did not explore the cause of the abnormal DPOAE readings. [9]

While there are many accepted causes of sensorineural hearing loss, such as noise exposure, and neuropathy, one of the most intriguing possible factors in patients with HIV is ototoxicity. In 1997, the World Health Organization published a report that defined ototoxicity as any damage caused to the organs of hearing and balance by drugs. The report goes on to state that cochlear ototoxicity tends to present as tinnitus, followed by a high frequency hearing loss. The patient might not notice these losses until they reach a threshold of >30dBHL. [10] Some investigators postulate that a common class of antiretroviral drugs called nucleoside reverse transcriptase inhibitors (NRTIs) may be responsible for sensorineural hearing loss caused by ototoxicity. NRTIs inhibit HIV's replication process by competing with the virus for the attention of reverse transcriptase enzymes. Once the NRTIs are incorporated into the cell DNA, they send signals that shut down the transcription mechanisms. While this stops HIV from using the cell to replicate itself, it also stops the production of the enzyme responsible for basic cell functions. [11]

One type of cell organelle affected by this shut down is the mitochondrion. The interruption in enzyme production affects the mitochondria's ability to synthesize mitochondrial

DNA (mtDNA). [12] Mitochondria are organelles responsible for energy production. If the mitochondria are unable to correctly produce the energy molecule adenosine triphosphate (ATP), the cell might not receive enough ATP to function correctly. [13] Henderson et al. (2006) reports that it is possible that metabolic stress due to insufficient energy production can case damage to cochlear hair cells. [14] While the exact cause of mitochondrial disease is difficult to determine, there is significant evidence of involvement of the cochlea. Mitochondrial deafness is almost always sensorineural, progressive, and symmetrical. It occurs at high frequencies, and is often exacerbated by noise exposure. [13]

The combination of the above factors, especially the possible relationship between sensorineural hearing loss and NRTI use, has led several scientists to begin research on the ototoxicity of NRTIs. Our limited ability to pinpoint the cause of mitochondrial deafness has led to speculation of contributing factors that increase the likelihood of sensorineural hearing loss in patients receiving HAART.

This systematic review will examine studies concerned with the ototoxicity of four antiretrovirals administered to HIV-positive patients: 1) didanosine, 2) stavudine, 3) lamivudine, and 4) zidovudine. While other antiretrovirals have been associated with ototoxicity, these four drugs are still commonly used in HAART. The following studies examine several scenarios involving ototoxicity alone, and ototoxicity in conjunction with age and/or noise exposure in order to investigate the possibility of ototoxicity associated with NRTI use.

Chapter 2

Review of the Literature

Method

Due to the limited amount of available research, subject selection criteria for this review were broad. This review included both human and animal studies. Human studies consisted of individual case studies and group studies. While preference was given to experimental studies, the inclusion of case studies was necessary due to the paucity of research in this area. The number of participating subjects ranged from 1 to 99. Age, ethnicity, and geographic location were not restricted.

Restricted criteria included study publication date and drug type. Studies published before 1995 were excluded from this study because most NRTIs were approved for use in or before that year. Research prior to 1995 focused on drug development and the most pressing side effects, while current research has focused more on quality of life issues. Drug type was restricted to ensure that studies focused on the most commonly administered NRTIs: didanosine, lamivudine, stavudine, and zidovudine. While there have been cases of permanent hearing loss associated with the ototoxic effects of the NRTI zalcitabine, [15] studies on the effects of zalcitabine were not included in this review because of the recent decline in zalcitabine prescriptions. The complexity of drug regimens rendered it necessary to include studies that accepted patients with a multi-drug therapy regimen; as a result, many of the patients detailed in the following studies were not taking the aforementioned drugs exclusively.

In order to obtain the most relevant research, database was not restricted. Searches were performed within the Medline and ComDisDome databases, but did not yield relevant results.

Most studies were selected after extensive hand searching. The primary source used in collecting the reviewed studies was the book *HIV/AIDS Related Communication Disorders,* which was compiled in 2010 by De Wet Swanepoel and Brenda Louw. Background and supporting information was obtained by hand searching the references used in the reviewed studies. Initial searches yielded about fifty articles, but ultimately, only eight articles met the aforementioned criteria.

While the initial purpose of this review was to examine the effects of NRTI use on hearing, it is impossible to ignore the affects of complex physiological and chemical interactions. As a result, several investigators took a holistic approach when researching the ototoxic effects of NRTIs. Based on the information obtained during the research process, included studies are divided into two categories: hearing loss caused by ototoxicity alone, and ototoxicity concomitant with other factors.

Results

Toxicity

The first two studies in this review concern children born to HIV-positive mothers. Children of HIV-positive mothers are usually exposed to antiretrovirals in one of two ways. One way is an HIV-positive woman may take antiretrovirals before she is aware of the pregnancy. The second common method of exposure occurs when doctors administer prophylactic doses of antiretrovirals to a pregnant mother in order to reduce the risk of virus transmission during childbirth, or the development of the disease in early childhood. Occasionally, these prophylactic treatments will be administered directly to the child postnatally. While there is no dispute over the effectiveness of these treatments, there are concerns about the potential side effects when these drugs are administered to infants and young children.

This concern led Poblano, Figueroa, Figueroa-Damien, and Schnass to examine the effect of prenatal exposure to zidovudine and lamivudine on brainstem auditory evoked potentials (BAEP). Subject selection for this study was complicated because the investigators had to find mother/infant pairs where both individuals met all inclusion criteria. Only mothers with low-risk pregnancies and generally healthy infants were included in the control and experimental groups. The investigators identified thirty-seven suitable mother/infant pairs. The assembled control group consisted of thirty-seven full-term infants selected based on head circumference. [16] This choice was justified by the results of a study by Rothenberg, Poblano, and Schnass, in which the authors reported cephalic perimeter to be a covariant of BAEP. [17]

Twelve of the infants in the experimental group were exposed to only zidovudine, while the other fifteen infants were exposed to a combination of zidovudine and lamivudine. All measurements were made in the neonatal period, and fell within the expected laboratory ranges

for BAEP measurements. While none of the measurements were outside of expected range, the investigators noted a statistically significant increase in the latency of the I-III waveforms of the exposed infants, compared to those of the control group. The type of drug therapy each exposed infant received was not significantly related to the BAEP readings of the exposed infant. Poblano et al. concluded that prenatal exposure to antiretroviral drugs subclinically affected the development of auditory regions in the brainstem. At the end of the study, none of the infants exposed to antiretrovirals demonstrated a clinical hearing loss, but the long-term effects of these subclinical developmental abnormalities remain unknown. [16]

The strengths of this study include careful subject selection and blinding of results. The scientists responsible for administering the BAEP tests did not know the exposure status of the infants. This practive prevented bias in interpreting the results of each infant's BAEP test. The investigators found no statistically significant differences between the experimental group and control group in gestational age, Apgar score, and age of BAEP testing. The similarity of infants in both groups eliminates confounding variables and lends credibility to the results. Blinding the results of the BAEP testing further strengthens the study.

Despite these strengths, there were weak points in the study. Poblano et al. acknowledged that the small sample size and short follow-up period prevented the formulation of a strong conclusion. They suggested that subsequent studies should obtain more subjects and follow subjects longer postnatally to examine potential effects of the subclinical differences in the auditory pathway. Another aspect of this study that can be improved is the way the investigators monitor prenatal levels of antiretrovirals. Determining the degree of exposure needed to produce subclinical abnormalities would have strengthened the study.

While the previous study followed infants with prenatal exposure to antiretrovirals, it is also important to examine the effects antiretrovirals might have on young children taking the drugs. Christensen et al. published a case study that followed a young girl from birth to 43 months of age. [18] The subject's mother was HIV-positive, but there is no information about possible prenatal exposure to antiretrovirals. The subject was prescribed the prophylactic bactrim, but her parents reported inconsistent administration. At one year of age, the subject was classified as HIV-positive with decreased immune function and no significant audiologic history. The subject was placed on zidovudine and didanosine in December 1995 at two years of age. The subject's adherence to the treatment regimen was sporadic until the subject moved in with her grandmother in fall 1996.

The subject's first evaluation occurred when she was 21 months old, during the period of intermittent treatment adherence. The subject did not tolerate earphones and could not be conditioned for play audiometry, so the investigators used visual reinforcement audiometry (VRA) to measure behavior response thresholds. The thresholds were measured within normal limits. The child was sedated during testing of transient otoacoustic emissions (OAEs). OAEs were present at 800-4000 Hz, with a response level of 18.8 dB in the left ear, and 21.1 dB in the right ear.

When the subject was evaluated at 34 months, her grandmother reported consistent use of zidovudine and didanosine. The subject continued to resist play audiometry conditioning and refuse headphones. Only ABR testing was performed due to time constraints. The subject's wave V latencies showed a significant decrease from the first evaluation, indicating a developing high frequency hearing loss.

In the third evaluation, performed at 43 months, the child had continued regular use of didanosine and zidovudine, but showed signs of possible viral progression. This could indicate that the antiretroviral therapy was no longer effective. The subject continued to refuse headphones and resist conditioning. VRA detected a hearing loss at 4 kHz with a threshold of 40dB. The researchers were unable to obtain OAEs due to lack of cooperation from the subject. Auditory brainstem response (ABR) testing showed that wave V latency continued to decrease. ABR was normal at .5 kHz, but showed no response at 8.5 kHz up to a threshold of 45 dB. There was a robust response at 75 dB. The subject awoke before an exact threshold could be determined, but a normal tympanogram indicated that the subject's high frequency hearing loss was sensorineural. [18]

This study is unique in that the investigators were able to follow the child from initiation of antiretroviral therapy, providing a thorough look at the deterioration of the child's hearing. Although the investigators were unable to use pure tone audiometry, the results can be considered reliable due to the consistent use of ABR at each testing session. In the future, it would be preferable to obtain a pure-tone audiogram so that the child is unable to rely on the use of the good ear during testing.

While the previous studies investigated the effects of young children exposed to HIV, Rey et al examined ototoxicity in a previously healthy young woman exposed to antiretrovirals. [18] They reported on a case of severe ototoxicity in a 23-year-old female medical student. The medical student was exposed to HIV while working on a patient with poor adherence to a highly active antiretroviral (HAART) routine. The student was given a prophylactic treatment of stavudine, lamivudine, and nevirapine. Less than two weeks after conclusion of treatment, the subject reported a sudden bilateral hearing loss with associated dizziness and tinnitus.

The student was examined, and an audiogram showed that the subject had a bilateral sensorineural hearing loss, predominantly between 2 and 4 kHz. The subject's CT and MRI were normal. An ABR test showed a moderately decreased left latency for wave V. The student was treated with the steroid prednisolone for ten days, but showed no change. Several months after the initial prophylactic treatment, there was a slight improvement when the student received treatment with clonazepam and vestibular reeducation sessions. The student remained negative for HIV. Neuroradiological and otological examinations revealed neurologic damage to the inner ears. After ruling out any other factors, the investigators concluded that stavudine was the likely cause of the student's hearing loss. [19]

The strength of this case study is the health of the subject. The typical antiretroviral patient has a severely compromised immune system. Even if an HIV patient is considered relatively healthy, the body's defenses are still impaired. The medical student in this case study reported no serious illnesses that would act as confounding variables. Furthermore, due to the sensitivity of the situation, the student's progress was closely monitored from the time of exposure. This ensured that any change in the student's status would be noted and documented, which provided the investigators with a comprehensive view of the nature of the toxicity.

The subjects in the previous studies appear to have irreversible effects from the ototoxic effects of antiretrovirals. In a case study by Colebunders, Depraetere, Wanzeele, and van Gehuchten, the subject's hearing returned to normal levels after discontinuation of the drug. [20]

The subject was a 37-year-old, homosexual Belgian man who began treatment with zidovudine in 1990. Zidovudine was replaced with didanosine in 1992. The subject was hospitalized with an infection in February 1996 and antibiotics were administered. In May 1996, the subject developed bilateral deafness. Tests showed normal bilateral otoscopy and normal

tympanometry. There was evidence of 40-60 dBhl bilateral sensorineural hearing loss. ABR testing showed increased bilateral latency of wave V, and an increased I-V interval. Didanosine was discontinued and audition returned to normal in August 1996. [20]

Despite the seemingly dramatic results, this study is severely limited. The report did not include a complete case history. The investigators used appropriate tests to assess the subject's condition, but, as the subject was never re-challenged with didanosine, the exact cause of the subject's sudden hearing loss remains unclear.

Toxicity Concomitant with Age and/or Noise Exposure

In 2008, Bektas, Martin, Stagner, and Lonsbury-Martin used DPOAE and auditory brainstem response (ABR) testing to measure the function of outer hair cells in mice treated with a combination of zidovudine and lamivudine. [21] The experimental group consisted of ten, eight-week-old female mice from the same commercial strain. Ten mice of the same age and strain made up the control group. The mice were given a combination of zidovudine and lamivudine orally by dissolving the drugs in drinking water. In phase 1, the experimental group received a twelve-week course of antiretroviral therapy, and DPOAE and ABR testing was performed weekly. After twelve weeks of drug administration and testing, the investigators concluded that t 1) there was no significant difference in hearing levels between the two groups of mice at the beginning of the study, and 2) the mice receiving the antiretrovirals showed no measurable change in DPOAE results.

In the second phase of this experiment, the mice were maintained on the drug regimen for one week, and then exposed to a 10kHz octave-band noise. DPOAE results were collected immediately after noise exposure, two days after exposure, and two weeks after exposure.

Immediately following noise exposure, the both groups presented with reduced measurements of DPOAEs at all levels. By the second day post-exposure, both groups of mice showed similar substantial recovery of DPOAE responses. After two weeks, however, the experimental group continued to exhibit high frequency hearing loss, while the control group showed DPOAE levels consistent with baseline magnitudes. [21]

While the investigators in this study conducted a well-documented and controlled study, the experiment had several limitations. First, the sample size was relatively small. While caring for more mice would require more labor and funding, a larger sample size would increase the validity of the study. Another shortcoming of this study is the lack of blinding. Blinding the drug administration and testing scenarios would greatly increase the level of evidence this study provides.

Although the research done by Bektas et al. was performed on mice, the findings of that study are consistent with the findings of a study Simdon, Watters, Bartlett, and Connick conducted on humans in 2001. [22] Simdon et al. examined the cases of three HIV-1 positive men treated at an infectious disease clinic at the Denver Veteran Affairs Medical Center (DVAMC) between 1997 and 2000. All of the subjects had a history of noise-induced hearing loss from occupational noise exposure, and no history of opportunistic infections or family history of hearing loss.

The first subject in the study is a 53-year-old male diagnosed with HIV in 1987. The subject was on zidovudine monoptherapy from 1988-1996. The subject complained of tinnitus in 1991, and a noticeable decline in speech discrimination in 1993. Medical records from 1986-1996 revealed a progression from a mild to severe high frequency hearing loss. In December 1996, the subject's drug regimen was changed to didanosine, stavudine, and nevirapine. Hearing

tests showed a continued decline in hearing acuity through June 1997. At the end of the study, the subject reported that his profound hearing loss interfered with his ability to socialize.

Subject two was a 47-year-old male who first presented to the DVAMC in 1992. The subject had a history of a stable, mild hearing loss, and tinnitus since 1966. In 1996, the subject began a drug regimen that included indinavir, stavudine, and lamivudine. Though the subject showed improvement after two months, indinavir was removed from the ART regimen due to side effects. The subject continued to use stavudine, lamivudine, and various protease inhibitors for two years. After the subject reported hearing deterioration in 1997, testing revealed moderately severe bilateral hearing loss substantially greater than the results of the subject's last hearing examination in 1985.

The subject's ART regimen was changed again in September 1998 to include didanosine, stavudine, lamivudine, and other drugs. In January 1999, the subject reported peripheral neuropathy and severe tinnitus. His ART regimen was discontinued, and the peripheral neuropathy and tinnitus improved. When the subject was returned to a didanosine-free ART regimen in April 1999, the neuropathy and tinnitus returned in full force. In December 1999, testing revealed a severe hearing loss. Upon conclusion of the study, the subject wore bilateral hearing aids and reported intolerable tinnitus.

The final subject in this study was a 43-year-old male with a history of HIV for at least fourteen years. He first presented to the DVAMC in October 1998 as a treatment-naïve patient. The subject had a history of stable hearing loss in the left ear since the early 1970s. Initial ART included stavudine and lamivudine. Four months after beginning the new drug therapy, the subject reported tinnitus and a noticeable decrease in hearing acuity. Over the course of two months, the subject's hearing loss worsened, and his tinnitus progressed from intermittent to

consistent. One month after the subject's physician replaced stavudine with zidovudine, the subject reported some improvement of the hearing loss and tinnitus. Subsequent testing revealed a mild hearing loss in the right ear, and a moderately severe hearing loss in the left ear. These losses were unchanged during a two-month period, and continued to persist throughout the end of the study. [22]

This study is problematic for several reasons. First, it consists entirely of case studies. The limited number of subjects makes it hard to generalize the results. The level of evidence is lowered further by the lack of experimental data. The investigators did not have the ability to prescribe a uniform ART regimen to the subjects, or any method to control for preexisting noise exposure or hearing loss. Strengths of this study include the relatively similar age and occupations of the subjects, and the extensive medical histories provided by the DVAMC.

Another possible concomitant factor with ototoxicity is age. In 1997, Marra et al. performed a case control study to investigate the association between antiretrovirals and hearing loss in HIV-positive patients. [5] The study enrolled 99 HIV seropositive volunteers to undergo standardized interviews, medication reviews, and hearing tests. The same investigator was responsible for conducting all tests and interviews. Hearing tests consisted of air conduction screening, and all subjects with thresholds >25dB at 4 kHz in at least one ear were determined to have hearing loss. Using this method, 46 subjects were diagnosed with hearing loss. The investigators then analyzed the collected data and found a statistically significant association between hearing loss and antiretroviral use in subjects 35 and older. [5]

The main concern in this study is the complete lack of blinding. There is an increased risk of bias due to the fact that the same investigator conducted the medical history interviews, medication reviews, and hearing screenings. The study would be strengthened if a different

investigator administered each test. Another issue with this study is the wide variety of antiretroviral therapies administered to subjects. The only therapy-related selection criteria required subjects to have filled a prescription for any drug classified as ototoxic within six months. Even though Marra et al. thoroughly analyzed the collected data, the lack of continuity in treatment regimen severely limits interpretation of these results.

Schouten et al. conducted the only study in this review that did not find a relationship between NRTIs and ototoxicity. [23] The investigators conducted a prospective observational pilot study on a group of thirty-three antiretroviral naïve subjects being treated with didanosine and zidovudine. Exclusion criteria included hearing loss requiring hearing aids, and substance abuse.

Subjects underwent audiometric testing prior to initiation of the study, and at 16 and 32 weeks into the study. At each visit, the investigators measured the subjects' viral load, performed tympanometry, and air conduction screening. Bone conduction testing was performed as needed. At the first visit, the investigators established a baseline that was used to evaluate hearing levels at subsequent visits.

At the beginning of the study, twenty-two subjects had a hearing level greater than 25 dB at one or more frequencies, sixteen subjects had a hearing level greater than 40 dB (primarily at high frequencies), and seven subjects had a hearing level greater than 60 dB (again, primarily at high frequencies). Although most of these increased hearing thresholds occurred primarily at high frequencies, only 16 subjects reported a history of sound exposure, and 14 reported tinnitus.

Subject attendance at the 16- and 32-week follow up visits was sporadic, but those who reported for testing demonstrated a trend of improvement from baseline measurements. At the end of the study, the investigators found that regardless of age, history of noise exposure, or

baseline viral load, there were no statistically significant changes in hearing over the course of the study. [23]

As one of the only prospective studies available on this subject, the present study demonstrates a higher level of evidence than the case studies in this review. While most of the case studies receive a grade of 3 on the Oxford scale of evidence, this study is a randomized controlled study, and receives a grade of 1b. [24] The use of treatment-naïve patients and detailed audiometry lends credibility to the study, but there are several areas of concern. The small sample size, coupled with the lack of a control group makes generalization of the results particularly difficult. Furthermore, this small sample excluded subjects with a pre-existing hearing loss requiring hearing aids. This is problematic because of previous findings that subjects with a greater degree of pre-existing hearing loss are particularly susceptible to hearing loss when treated with NRTIs. The investigators were also unable to randomize the treatment regimens of study participants. Finally, the timeline for follow-up visits in this study was very short. If there are no detrimental side effects, the subjects of the study may stay on the prescribed drug regimens for months or years. The fact that this study was finished in less than a year leaves unanswered questions about the long-term side effects of didanosine and zidovudine.

Level of Evidence

As shown in the chart below, the overall level of evidence of the studies in this review is quite low; most of the studies only have a level of evidence grade of 3. [24] Although the original intent of this review was to obtain a higher overall level of evidence, only eight studies met the inclusion criteria. The qualifying studies had a tendency toward being descriptive, rather than experimental.

Study (by author)	Type of Study [24]	Level of Evidence [24]
Bektas[20]	Randomized controlled study	1b
Christensen[17]	Case study	3
Colebunders[19]	Case study	3
Marra[5]	Case control	3
Poblano[16]	Controlled study without randomization	2b
Rey[18]	Case study	3
Schouten[22]	Non-experimental study	3
Simdon[21]	Case Series	3

While some of the studies utilized control groups, [16, 21] there is an overall lack of blinding. In order to increase the level of evidence surrounding this topic, an effort should be made to design more blinded, experimental studies. While ethical constraints might prevent most researchers from conducting clinical trials, or randomized human studies, it is still possible to blind certain aspects of the studies. In the 2008 Bektas study, the investigators could have blinded the administration of antiretrovirals by housing all of the mice separately, rather than just the mice receiving antiretrovirals. [21] In human studies like the one conducted by Marra et al. in 1997, it is possible to blind results by using different investigators to conduct subject interviews and audiometric testing. These tasks can also be outsourced to qualified professionals not otherwise involved in the study.

Chapter 3

Discussion

Although the lack of randomized, controlled studies that qualified for this review was originally disappointing, it was not surprising. The low number of available studies indicates that research on the ototoxicity of NRTIs is still in the early stages. Although research will continue to progress, it is unlikely that the average study on the ototoxic effects of antiretroviral drugs will be a randomized, controlled trial. Aside from the ethical concerns of randomizing the distribution of drugs to critically ill patients, there are several obstacles that prevent investigators from determining the exact ototoxic effects of antiretroviral drugs.

First and foremost of these obstacles is the complexity of the ART regimens. The United States Food and Drug Administration (FDA) has approved twenty-three single drugs from six different drug classes for the treatment of HIV-positive patients. The FDA has also approved the use of eight fixed-dose combination drugs, which include drugs from two or more classes in one dose. [25] In the United States, ART regimens are normally decided upon after careful consideration of the patient's history, current medication the patient is taking, and side effects of the ART medication. Most of these regimens include two or three antiretrovirals, complicating researchers' ability to determine the cause of hearing loss. [26]

These complex drug interactions are further complicated by the tendency of HIV patients to require extensive medical care, due to their weakened immune systems. HIV patients are highly susceptible to opportunistic infections that must be treated with ototoxic antibiotics. For example, aminoglycosides are commonly prescribed to treat diseases such as tuberculosis, and are a known toxin for the hair cells in the inner ear. [27] It is postulated that interactions between two ototoxic drugs may increase the likelihood of hearing loss. This problem is of particular

concern in countries like South Africa, where patients receiving high-dose antiretroviral medications are exposed to highly infectious diseases like tuberculosis. [28] This example shows that while the complexity of drug regimens must be accounted for, study design is also affected by demographics.

While the mechanism of HIV/AIDS infection is the same across the globe, there are distinct differences in the affected populations of different countries. For example, a 2008 WHO report estimated that in Swaziland, between 20% and 37% of young adults aged 15-24 years are infected with HIV/AIDS, and the adult HIV rate was estimated to be about 26%. [29] In the United States, less than 2% of young adults age 15-24 were living with HIV/AIDS in 2008, while the adult infection rate was only 0.6%. [30] Age is an important demographic factor because older HIV patients are more likely to have a history of hearing loss. [5]

Other demographic considerations include income, access to healthcare, and availability of treatment. The aforementioned WHO reports show large disparities between the United States and Swaziland in all three of these categories. These gaps are consistently present in comparisons of a developed nation to a developing nation. While American HIV patients have access to complex drug regimens and advanced audiological services, most African patients are prescribed high doses of low-cost drugs, like zidovudine, with little to no monitoring. [28] In 2009, Fagan and Jacobs conducted a survey of the availability of otolaryngology, audiology, and speech therapy services in eighteen Sub-Saharan countries, compared to the availability of the same services in the United Kingdom. The investigators found that all of the African nations reported poor access to the most basic audiologic services, including screening and hearing aids. [31] If studies are conducted in developing countries, the lack of audiology facilities might result in trouble obtaining high frequency audiometric data, and sporadic subject attendance. [28]

Regardless of these barriers, it is important that research on the ototoxicity of antiretrovirals continues to advance. As treatment regimens become more effective, HIV patients are living longer, but are beginning to suffer from side effects of their medication. This change in the needs of HIV patients allows researchers to shift their focus from merely keeping patients alive to improving quality of life. The studies reviewed in this report suggest two areas of concern that scientists should continue to investigate.

The possible correlation between age, noise exposure, and hearing loss in patients treated with NRTIs is especially relevant in highly industrialized countries, where HIV patients are more likely to survive to old age. Research in these countries might include identification of at-risk populations, and investigation into more effective hearing protection protocols. For example, members of the U.S. military who have been exposed to HIV are also likely to have a history of noise exposure. These patients should be counseled on ways to protect their hearing during their term of service, and after return to civilian life. Further research into the relationship between NRTI ototoxicity and concomitant factors will increase scientific knowledge that can be used for awareness campaigns and patient education.

Prenatal exposure to potentially ototoxic NRTI regimens is another area of concern. While transmission of HIV from mother to child is a global problem, children born in developed countries are less likely to contract the virus, and therefore are exposed to fewer antiretrovirals. Meanwhile, developing countries have a much higher incidence of childhood HIV infection. It is important for researchers in these countries to examine the long-term effects of NRTI exposure on a child's auditory system.

While regional differences in populations affected by the disease complicate efforts to conduct research on NRTI ototoxicity, they also encourage global collaboration. The above

suggestions of regional research interests are by no means exclusive. Children in the USA are just as susceptible to hearing damage as children in Chile or Botswana. Likewise, adults in developing countries may work around noisy machinery while taking NRTIs. If researchers continue to conduct ototoxicity research relevant to the unique HIV-positive populations of their home countries, the knowledge gained through these endeavors may be applied to other HIV-positive populations. Global collaboration will be essential not only in determining the risk NRTIs pose to hearing, but in addressing, and eventually solving, the potential auditory side effects faced by millions of HIV patients worldwide.

Currently, one has to conclude that the evidence that NRTIs affect hearing is inconclusive. Most hearing losses that have been associated with NRTI use are characterized as high frequency sensorineural losses. While there have been reports of concomitant factors like age and noise exposure increasing the likelihood of hearing loss in patients, there has been no definitive information to explain the cause of these losses. Further research is needed to determine the full effects of NRTIs on hearing and the auditory system.

References

1. World Health Organization. (2011). Global summary of the AIDS epidemic. Retrieved from http://www.who.int/hiv/data/2012_epi_core_en.png.

2. Alimonti, J., Ball, T.B., Fowke, K.R. (2003). Mechanisms of $CD4^+$ T lymphocyte cell death in human immunodeficiency virus infection and AIDS. *Journal of General Virology, 84*(7), 1649-1661.

3. AIDS.gov. (2010). *CD4 count.* Retrieved from http://aids.gov/hiv-aids-basics/just-diagnosed-with-hiv-aids/understand-your-test-results/cd4-count/.

4. Moazzez, A., Alvi, A. (1998). Head and neck manifestations of AIDS in adults. *American Family Physician, 57*(8), 1813-1822.

5. Marra C.M., Wechkin H.A., Longstreth W.T. Jr., Rees T.S., Syapin C.L., Gates G.A. (1997) Hearing loss and antiretroviral therapy in patients infected with HIV-1. *Archives of Neurology, 54*, 407–410.

6. McNaughten AD, Wan PT, Dworkin MS. (2001) Correspondence: prevalence of hearing loss in a cohort of HIV-infected patients. *Archives of Ototlaryngology, Head and Neck Surgery, 127*, 1516-1518.

7. Zuniga, J. (1999) Communication disorders and HIV disease. *Journal of the International Association of Physicians in AIDS Care,* 16-23. Retrieved from http://www.thebody.com/content/art12344.html.

8. Chao, C.K., Czechowicz, J.A., Messner, A.H., Alarcon, J., Roca, L.K., Rodriguez, M.M.L., Villafuerte, C.G., Montano, S.M., Zunt, J.R. (2011) High prevalence of hearing impairment in HIV-infected Peruvian children. *Otolaryngology-Head and Neck Surgery, 146(2)*, 259-265.

9. Ranjan, R., Bhat, J.S. (2008). DPOAE in HIV infected adults. *Online Journal of Health and Allied Sciences, 7*(1), 1-5.

10. Smith, A., Mackenzie, I. (1997) Hearing loss from ototoxics. *WHO Drug Information, 11*(1), 7-10.

11. Brinkman, K., Kakuda, T.N. (2000) Mitochondrial toxicity of nucleoside analogue reverse transcriptase inhibitors: a looming obstacle for long-term antiretroviral therapy. *Current Opinion in Infectious Diseases, 13,* 5-11.

12. Birkus, G., Hitchcock, M.J.M., Cihlar, T. (2001). Assessment of mitochondrial toxicity in human cells treated with tenofovir: comparison with other nucleoside reverse transcriptase inhibitors. *Antimicrobial Agents and Chemotherapy*, 46(3), 716-723.

13. Kokotas H, Petersen MB, Willems PJ. (2007). Mitochondrial deafness. *Clinical Genetics, 71*, 379–391.

14. Henderson, D., Bielefeld, E.C., Harris K.C., Hu B.H. (2006). The role of oxidative stress in noise-induced hearing loss. *Ear and Hearing, 27,* 1-19.

15. Monte, S., Fenwick, J.D., Monteiro, E.F. (1997). Irreversible ototoxicity associated with zalcitabine. *International Journal of STD & AIDS, 8*, 201-202.

16. Poblano, A., Figueroa, L., Figueroa-Damien, R., Schnaas, L. (2004). Effects of prenatal exposure to zidovudine and lamivudine on brainstem auditory evoked potentials in infants from HIV-infected women. *Proceedings of the Western Pharmacology Society, 47*, 46-49.

17. Rothenberg, S.J., Poblano, A., Schnass, L. (2000). Brainstem auditory evoked potential at five years and prenatal and postnatal blood lead. *Neurotoxicology and Teratology, 22*(4), 503-510.

18. Christensen, L.A., Morehouse, C.R., Powell, T.W., Alchediak, T., Silio, M. (1998). Antiviral therapy in a child with pediatric human immunodeficiency virus (HIV): case study of audiologic findings. *Journal of the American Academy of Audiology, 9*, 292-298.

19. Rey, D., L'Heritier, A., Lang, J.M. (2002) Severe ototoxicity in a health care worker who received postexposure prophylaxis with stavudine, lamivudine, and nevirapine after occupational, exposure to HIV. *Clinical Infectious Diseases, 34*, 418-419.

20. Colebunders, R., Depraetere, K., (1998). Deafness caused by didanosine. *European Journal of Clinical Microbiology & Infectious Diseases, 17*, 214-215.

21. Bektas, D., Martin G.K., Stagner, B.B., Lonsbury-Martin, B.L. (2008). Noise-induced hearing loss in mice treated with antiretroviral drugs. *Hearing Research, 239*(1-2), 69-78.

22. Simdon, J., Watters, D., Barlett, S., Connick, E. (2001). Ototoxicity associated with use of nucleoside analog reverse transcriptase inhibitors: a report of 3 possible cases and review of the literature. *Clinical Infections Diseases, 32*, 1623-1627.

23. Schouten, J.T., Lockhart, D.W., Rees, T.S., Collier, A.C., Marra, C.M. (2006). A prospective study of hearing changes after beginning zidovudine or didanosine in HIV-1 treatment-naïve people. *BMC Infectious Diseases*, 6, 28.

24. American Speech-Language Hearing Association. (2013). Step 3: assessing the evidence. Retrieved from http://www.asha.org/members/ebp/assessing.htm.

25. AIDSinfo. (2012). FDA-approved anti-HIV medications. Retrieved from http://aidsinfo.nih.gov/contentfiles/ApprovedMedstoTreatHIV_FS_en.pdf.

26. AIDSinfo. (2012). Recommended HIV treatment regimens. Retrieved from http://aidsinfo.nih.gov/contentfiles/RecommendedHIVTreatmentRegimens_FS_en.pdf.

27. Ryback, L.P., Ramkumar, V. (2007). Otoxicity. *Kidney International, 72*, 931-935.

28. Khoza-Shangase, K. (2010). Is there a need for ototoxicity monitoring in patients with HIV/AIDS. *African Journal of Pharmacy and Pharmacology, 4*(9), 574-579.

29. World Health Organization. (2008). Epidemiological fact sheet on HIV and AIDS core data on epidemiology and response: Swaziland. Retrieved from http://apps.who.int/globalatlas/predefinedReports/EFS2008/full/EFS2008_SZ.pdf.

30. World Health Organization. (2008). Epidemiological fact sheet on HIV and AIDS core data on epidemiology and response: United States of America. Retrieved from http://apps.who.int/globalatlas/predefinedReports/EFS2008/full/EFS2008_US.pdf.

31. Fagan, J., Jacobs, M. (2009). Survey of ENT services in Africa: need for a comprehensive intervention. *Global Health Action, 2,* included supplements.