Audiologic Monitoring for Potential Ototoxicity in a Phase I Clinical Trial of a New Glycopeptide Antibiotic

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Abstract

This study describes audiologic methodology and results for evaluating potential ototoxicity in a phase I clinical trial of a new glycopeptide. This study was conducted under good clinical practices, which are regulated by the US Food and Drug Administration (FDA) (21 Code of Federal Regulations), and input from the FDA was sought prior to study implementation. Healthy, normal volunteers underwent extensive medical and audiologic assessments as part of this phase I dose-escalation study of dalbavancin, a new glycopeptide, to assess potential side effects. Audiologic monitoring included air-conduction thresholds in the conventional (0.25-8 kHz) and high-frequency (10-16 kHz) ranges. At baseline, subjects were also tested using word recognition, bone conduction testing if indicated, and tympanometry. Full testing was to be repeated if any subject met the American Speech-Language-Hearing Association (ASHA) 1994 criteria for ototoxic change. However, no subjects demonstrated ototoxic change after receiving dalbavancin, nor were any false-positive results obtained.

Key Words: Audiologic monitoring, clinical trials, dalbavancin, glycopeptide, high-frequency audiometry, ototoxicity monitoring, phase I, FDA

Abbreviations: CRF = case report form; DHI = Dizziness Handicap Inventory; EHFI = Extended High Frequency; FDA = US Food and Drug Administration; GCPs = good clinical practices

Sumario:

Este estudio describe la metodología audiológica y los resultados de una evaluación por ototoxicidad potencial en el ensayo clínico fase I de un nuevo glucopéptido. Este estudio fue conducido bajo buenas prácticas clínicas, las cuales son reguladas por la Administración de Alimentos y Drogas de los Estados Unidos de América (FDA) (Código 21 de Regulaciones Federales), y se buscó orientación por parte de la FDA antes de la implementación del mismo. Se sometió a un grupo de voluntarios saludables y normales a una evaluación extensiva de tipo médico y audiológico como parte de esta fase I de un estudio de incremento en la dosis de dalbavancina, un nuevo glucopéptido, para evaluar efectos secundarios potenciales. El monitoreo audiológico incluyó umbrales de conducción aérea en el rango convencional (0.25-8 kHz) y de alta frecuencia (10-16 kHz). Al nivel basal, los sujetos tam-
Protocols for ototoxicity monitoring in the clinical setting have been explored and developed over the last few decades. However, no guidelines for ototoxicity monitoring have been specifically reported for use in clinical trials for new drugs. Clinical trials are essentially methods for testing new therapies to ensure that they are both safe and effective. Phase I studies are specifically designed to test a new drug or other therapy to determine a safe dosing range, detect potential side effects, and to determine pharmacokinetics. Phase I studies are generally conducted on a relatively small group of 20 to 100 subjects using a dose-escalation study design. Typically, in a phase I study, subjects are normal, healthy, paid volunteers. Phase II clinical trials evaluate the study drug or therapy in a larger group to test its efficacy for its therapeutic purpose and to further determine safety. Therefore, phase II trials are usually conducted in the target patient populations. Phase III trials evaluate the study drug or treatment in larger groups of patients to confirm its effectiveness, and compare it to other treatments that constitute the standard of clinical care. Any further information needed to ensure that the drug can be used safely is also gathered and analyzed during the phase III trial. Phase IV trials are performed after the drug or treatment has been marketed to evaluate possible side effects in a variety of populations and particularly to evaluate any side effects associated with long term use.

In this study, a new glycopeptide antibiotic, dalbavancin, was evaluated in a phase I clinical trial. Evaluation of the safety, pharmacokinetics, and pharmacodynamic assessments for this trial are published separately (Leighton et al 2001, Leighton et al submitted for publication). Although vancomycin is the only glycopeptide currently licensed in the United States, dalbavancin has the potential advantages of ease of use (i.e., once-weekly as opposed to twice-daily administration) and increased potency (i.e., 1 g/week vs 2 g/day).

Vancomycin was first introduced in 1958 and is well established in clinical practice. Because of the “mycin” suffix, vancomycin is sometimes confused with the aminoglycosides, such as gentamicin, amikacin, tobramycin, and kanamycin. Both drugs are given parenterally for serious infections in hospitalized patients. Aminoglycosides are generally used for gram-negative infections, whereas glycopeptides are generally prescribed for drug-resistant gram-positive infections. Both drugs are sometimes given together if a mixed infection is suspected.

Although aminoglycoside ototoxicity is clearly established (see review by Forge and Schacht, 2000), controversy exists as to whether and to what extent vancomycin causes ototoxicity. Animal study data are conflicting, although the great majority of studies suggest no ototoxicity (Brummett, 1981; Brummett, 1993; Freeman et al, 1993). The differences that do exist between
results and interpretations among studies cannot be easily attributed to species, agent, or dosing differences. Clinical data are also conflicting (Brummett and Fox, 1989; Freeman et al, 1993; Brummett, 1993; Garrelts et al, 1994; Cantu et al, 1994; Murphy and Pinney, 1995; Barth and DeVincenzo, 1996; Wood, 1996; Elting et al, 1998; Schaefer et al, 1999; Begg et al, 2001; Machado et al, 2001). Prospective clinical studies generally show no glycopeptide ototoxicity unless a prior or concomitant ototoxin was administered. For example, many of these patients had prior aminoglycoside administration (Brummett and Fox, 1989; Brummett, 1993; Freeman et al, 1993; Elting et al, 1998).

Aminoglycosides can be retained in the cochlea for at least 11 months after discontinuation and are known to cause delayed hearing loss (Aran, 1995). Consequently, when vancomycin is administered after treatment with aminoglycosides, hearing loss occurring during vancomycin administration could be secondary to delayed-onset aminoglycoside ototoxicity. It is also possible that vancomycin may exacerbate aminoglycoside-induced hearing loss, but reports are conflicting (Mellor et al, 1985). Another consideration when attempting to interpret the literature is that many patients treated with vancomycin had other medical factors, which may have caused or contributed to hearing loss. In some clinical reports, no baseline hearing assessment was obtained prior to vancomycin administration. Considering that over 10 percent of the U.S. population is hearing impaired, interpreting hearing loss causality without baseline data is problematic. In some cases, “possible” ototoxicity was reported, but no actual testing was performed. It is interesting to note that no vancomycin ototoxicity was found even in a 1998 prospective study of peritoneal dialysis patients (Gendeh et al, 1998). Keller and colleagues (1994) did report ototoxicity in 1 of 39 patients receiving vancomycin, but that patient was also receiving furosemide, which could have caused the hearing loss.

Nonetheless, vancomycin ototoxicity cannot be ruled out. If it does occur, it is extremely rare and/or may be related to overdosing (Matzke et al, 1986; Brummett and Fox, 1989; Brummett, 1993; Garrelts et al, 1994; Cantu et al, 1994; Gendeh et al, 1998; Schaefer et al, 1999). However, Gilbert and colleagues (1994) reported no ototoxicity even at high peak concentration levels. Excellent reviews of the literature through 1994 exist (Brummett and Fox, 1989; Brummett, 1993; Cantu and Yamanka-Yuen, 1994).

Unlike platinum-based chemotherapeutics and aminoglycosides, no national audioligic monitoring guidelines for vancomycin or other glycopeptides have been published (American Speech-Language-Hearing Association [ASHA], 1994). Clinically, vancomycin is not usually monitored for ototoxicity. However, this glycopeptide is widely suspected of causing ototoxicity, and ototoxicity is listed as a potential side effect in the literature. Therefore, for this dose-escalation phase I trial of a new glycopeptide, both auditory and vestibular function were included in the study assessments to assess the potential of dalbavancin to cause ototoxicity.

First, an appropriate ototoxicity monitoring protocol for this phase I study needed to be developed. Various protocols have been described for auditory ototoxicity monitoring in clinical populations (Campbell and Durrant, 1993; Fausti et al, 1999). ASHA also has provided “recommended procedures” in their document, “Guidelines for the Audioligic Management of Individuals Receiving Cochleotoxic Drug Therapy” (1994). In many clinical settings, pure tone audiometry is conducted in the conventional frequency range (250–8000 Hz) as a means of monitoring for ototoxicity. However, the conventional frequency range alone may not detect early ototoxic changes (Fausti et al, 1984a, 1984b; Rappaport et al, 1985; Kopelman et al, 1988).

The frequency range above 8000 Hz through 20000 Hz, is referred to as the extended high-frequency (EHF) range. Over the last four decades, EHF testing, or high-frequency audiometry, has been proven usually to detect ototoxic changes early, before the conventional frequency range, and thus communicative function is affected (Jacobson et al 1969; Fausti et al, 1984a, 1984b; Rappaport et al, 1985; Kopelman et al, 1988). Using this frequency range generally detects cochlear hair cell changes early because most ototoxic changes occur primarily in the basal end of the cochlea, which is most sensitive to high
frequencies. Although research on high frequency audiometry has been conducted for several decades (Fletcher, 1929, 1965; Rosen et al., 1964; Zilis and Fletcher, 1966), initial clinical acceptance of high-frequency audiometry was slow. Initially, EHF equipment was not commercially available, and no standard calibration references existed. As reviewed by Northern and Ratkiewicz (1985), attempts to establish high-frequency normative data revealed wide differences across normal subjects for thresholds. However, when using the EHF range for ototoxic monitoring, the inter-subject variability at baseline does not pose a significant problem because comparisons are based on each individual’s EHF thresholds obtained over a relatively short period of time and compared with baseline thresholds for that particular subject. Thus, whether one’s EHF thresholds are normal or impaired, compared with a standardized reference population, is essentially irrelevant to ototoxicity monitoring. High intrasubject variability, most commonly demonstrated as poor test-retest reliability, would pose a significant deterrent to using this range for ototoxicity monitoring. However, intrasubject variability over time using standardized clinical procedures and commercially available equipment no longer appears to be an issue (Fausti et al., 1985; Frank 1990, 2001).

There are no generally recommended procedures for vestibular ototoxicity monitoring, although vestibular disorders can occur secondary to certain drug exposures, particularly aminoglycosides (Black and Pesznecker, 1993). We selected the Dizziness Handicap Inventory (DHI) as a quick, noninvasive method of screening this parameter throughout the study. Although the DHI has not been previously used to monitor potential ototoxicity, it is the most widely used and best validated self-assessment scale for dizziness and dysequilibrium (Jacobsen et al., 1991; Fielder et al., 1996; Enloe and Shields, 1997; Cowand et al., 1998; Whitney et al., 1999; Jacobsen and Calder, 2000).

The purpose of this report is to describe the audiologic methods used and results of a phase I clinical trial testing a new drug for potential ototoxicity. The results of the trial are also presented. This study was conducted as part of the drug development process, and the results will be included in the submission of safety and efficacy data that is reviewed by the US Food and Drug Administration (FDA) before the drug can be marketed. Therefore, the sponsor sought input in the design of the trial from the FDA before implementing the protocol. The study was conducted under good clinical practices (GCPs), which are regulated by the FDA (21 Code of Federal Regulations). To our knowledge, this is the first report of its kind in the literature.

METHOD

The study was a randomized, double-blind, placebo-controlled dose-escalation study in healthy volunteers. This study was performed through the Clinical Research Center at UMDNJ-Robert Wood Johnson Medical School after approval by the Institutional Review Board at UMDNJ-Robert Wood Johnson Medical School. Audiologic data were collected at the Center for Speech and Hearing Sciences. The authors discussed various aspects of the ototoxicity monitoring methods with an FDA audiologist prior to implementation of the trial.

Study Population: Inclusion Criteria

To be eligible for participation in the study, each subject was to be a healthy male or a nonpregnant, nonlactating female who was using effective contraception, was surgically sterile, or was postmenopausal; was between the ages of 18 and 55 years; had given informed consent; and had a normal audiologic assessment at baseline consisting of symmetric hearing with air-conduction thresholds no worse than 25 dB HL for the frequencies 250 to 8000 Hz bilaterally, no threshold asymmetry ≥ 20 dB at any test frequency, and word recognition for W-22 recorded word lists was to be ≥ 90 percent bilaterally. Eligible subjects were not to have abnormal otoscopic findings, significant air-bone gaps, abnormal tympanograms, or other indications of middle ear abnormality; history of fluctuant hearing, persistent tinnitus, balance disorder, otologic surgery or disease, tumor of the head, neck, or
auditory system, head injury, Meniere’s disease, autoimmune inner ear disease, perilymphatic fistula, central nervous system disorder, or significant noise exposure; significant prior or current exposure to aminoglycoside antibiotics, chemotherapy, or current use of loop diuretics; known hypersensitivity or allergy to a glycopeptide antibiotic; history of alcohol or drug abuse; taking any aspirin or nonsteroidal anti-inflammatory drugs within one week of study entry; or inability to abstain from use of any drugs (oral contraceptives were not included in this criterion) or to avoid excessive noise exposure during the study.

Dosing Test Product, Dose, and Mode of Administration

Study drug (dalbavancin or placebo) was administered intravenously over 30 minutes, once in the single-dose cohort and daily for seven days in the multiple-dose cohort. In the single-dose cohort, the starting dose was 140 mg followed by 220 mg, 350 mg, 500 mg, 630 mg, 840 mg, and 1120 mg. Dose-escalation proceeded via a modified Fibonacci series up to 1120 mg. In a Fibonacci series, the last two numbers are added to obtain the next (0, 1, 1, 2, 3, 5, 8 etc.). A “modified” Fibonacci series means that the dose was escalated using this strategy until it empirically appeared to be too great, at which time smaller steps were introduced. A modified Fibonacci series is often used as a dose-escalation strategy in phase I clinical trials, with its purpose being to escalate to the maximum tolerated dose both quickly and safely (Ratan et al. 1993). In the multiple-dose phase of the study, dosing consisted of a loading dose (two equal doses given 12 hours apart) followed by a maintenance dose. The starting regimen was a loading dose of 300 mg given 150 mg every 12 hours followed by a maintenance dose of 30 mg per day for 6 days. Dose-escalation proceeded as follows: 400/40 mg, 600/60 mg, 800/80 mg, 1000/100 mg. For the single-dose protocol, three subjects received active drug at each level and seven subjects received placebo (one placebo subject for each level of drug). For the multiple-dose protocol, three subjects received active drug at each level and six subjects received placebo. The study design was selected to provide adequate statistical power while exposing as few subjects as possible to any potential risk in a phase I study of a new drug.

Auditory Monitoring

The following standardized procedures were performed for audiologic assessment. In clinical trials, protocols of new drugs criteria for determination of adverse events, including significant change criteria, must be clearly defined prior to study implementation. Adverse events suggestive of ototoxicity were monitored using the significant change criteria outlined below.

Test Schedule

At baseline, two audiologic assessments were conducted 24 hours apart on all subjects. The first baseline assessment occurred 2 days prior to drug or placebo administration (day-2) and the second 1 day prior to drug or placebo administration (day-1). Audiologic assessments were repeated on days 2, 7, and 14 in the single-dose cohort and on days 2, 7, 14, and 21 in the multiple-dose cohort.

Personnel

All testing was performed by audiologists having at least a master’s degree in audiology from a graduate program accredited regionally and by ASHA. The audiologist was responsible for all testing and for maintaining all audiologic data files for all patients enrolled in the study.

Equipment

All testing was conducted using a GSI 61 audiometer with the appropriate earphones for the study frequencies (3A inserts for 8 kHz and below, Sennheiser HDA 200 earphones for high-frequency headphones for 9 kHz and above) in a sound booth meeting American National Standards Institute (ANSI S3.1-1999) specifications. Test equipment, test environment, procedures, and personnel met all
relevant ASHA, American Academy of Audiology, and ANSI standards and guidelines. The audiometer was calibrated prior to study onset and every 6 months during the study according to ANSI 3.6 1996, including ANNEX C for the extended high-frequency range. Imittance testing was conducted with the GSI 38 immittance bridge. Prior to each day’s testing, a biological listening check was conducted for both the insert earphones and the high-frequency transducers.

**Audiologic Assessment**

Baseline audiometry (Days –2 and –1) included bilateral air-conduction pure-tone threshold audiometry in the conventional (0.25-8 kHz) range and high-frequency (9-16 kHz) range prior to study drug administration. Additionally, on day –2, bone-conduction thresholds, speech reception thresholds, tympanometry, and word recognition using recorded W-22 word lists at 25 dB HL were tested. Pure-tone threshold testing was conducted using the modified Hughson-Westlake procedure. Pure-tone air-conduction testing was conducted at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 11.2, 12.5, 14 and 16 kHz. Bone-conduction testing was conducted at 0.25, 0.5, 1, 2, 3, 4 kHz if the air-conduction threshold at that frequency was 15 dB HL or greater. On day –1, only pure-tone air-conduction testing was repeated at all air-conduction frequencies to determine that the subject provided reliable responses. Responses were considered reliable if retest thresholds at any frequency did not exceed ± 5 dB of the previously obtained threshold response. Subjects not meeting this criterion were eliminated from further audiologic study.

Bone-conduction thresholds, speech reception thresholds, and word recognition measures were not to be repeated at every subsequent assessment unless there was a significant change meeting criterion values that replicated within 24 hours. In that event, tympanometry to assess middle ear function was also to be repeated. Audiologic reassessment was repeated at 2, 7, and 14 days after drug initiation in the single dose cohort, and at 2, 7, 14, and 21 days after drug initiation in the multiple-dose cohort.

Comparisons of audiologic assessments at these visits were made to the baseline assessment, which was defined as the day –2 assessment.

Each audiologic assessment also included a vestibular assessment questionnaire (DHI), which each subject completed prior to undergoing the audiometry testing.

**Data Management**

Audiologic data were collected on a standard audiologic case report form (CRF) designed for the study. All CRFs were sent by patient name in inverse chronological order. Following completion of each dose level for each cohort, copies of all data were sent to the principal investigator and to the audiology advisor for the study. The audiology advisor was responsible for reviewing data for all subjects on an on-going basis. If a significant change in hearing was noted on any patient at any time during the study, and these results replicated 24 hours later, both the principal investigator and the audiology advisor were to be notified immediately and test results faxed to them. However, no significant change occurred for any subject, so this procedure was not implemented.

**Significant Change Criteria**

Ototoxicity was defined by the ASHA 1994 significant change criteria of either (1) ≥20 dB change at any frequency, (2) ≥10 dB change at any two adjacent frequencies, or (3) loss of response at three consecutive frequencies where responses were obtained at baseline (ASHA, 1994). To be considered significant for ototoxicity, these changes must have replicated 24 hours later, with no indication of middle ear abnormality. Subjects served as their own controls for ototoxic change, which was computed relative to baseline measures.

**Determination of Adverse Event**

Any audiologic test result meeting the significant change criteria described above was to be considered an ototoxic adverse
event. Any data from a subject with an adverse event were to be immediately unblinded with respect to drug regimen (study drug or placebo). However, an oto-toxic adverse event never occurred in any subject in this study, so this procedure was not implemented in the course of the study.

**Vestibular Monitoring**

In addition to the procedures described in Part 1, a DHI score was completed by each subject at baseline and each of the time points of audiometric assessment to screen for vestibular dysfunction. This questionnaire was administered just prior to each audiologic assessment in the study. The form was filed along with each audiologic assessment and served as the report form for this measure. The DHI constituted an integral part of the screening process. Any volunteer that answered the prescreen question with "yes" or "sometimes" would not be enrolled into the study. Because this was a prospective study of a new drug, shortened versions of this form were not used as they frequently are in clinical settings.

As per Jacobson and Newman (1990), a "yes" response is scored 4 points, a "sometimes" response is scored 2 points, and a "no" response is scored 0 points. Any subject receiving a score of 5 points or greater was to be referred to an otolaryngologist for full evaluation, but this never occurred in the course of the study.

**Statistical Analysis**

Auditory threshold data were analyzed using a multifactorial analysis of variance (ANOVA) with repeated measures. All analyses were conducted with powers of ≥ .80. Time interval [(5 levels) 2 prescreenings, days 2, 7, and 14 postadministration], ear (2 levels), and frequencies tested (13 levels) were treated as within-subject independent variables (all subjects received all levels of these variables). Treatment (15 dosing conditions) was treated as a between-subject independent variable (subjects received only one level of this variable). The dependent variable was the threshold (dB HL) measured under each of the above conditions. This study design allows for high statistical power for the major hypothesis of interest by using a large number of repeated observations on a relatively small number of subjects. The major hypothesis of interest was any change in threshold that may have occurred over the time intervals tested. The statistical term appropriate to test this hypothesis was treatment x frequency x time interval interaction if the change was different for frequency or the treatment x time interval interaction if the change was not different for frequency. In order to maximize the possibility of finding an effect, sphericity was assumed (homogeneity of covariance for the within-subject variables). When sphericity was not assumed, corrections (Greenhouse-Geisser or Huynh-Feldt) for violations of sphericity did not alter any statistical outcomes but did result in a loss of power.

**RESULTS**

**Subject Disposition**

Subject disposition, by treatment group, is presented, in Tables 1 and 2. One hundred sixty-two healthy adult subjects were screened. Of these, 52 subjects qualified and were enrolled. Forty-eight subjects were excluded because they did not meet protocol-defined audiology screening criteria. The remaining 62 subjects were excluded for a variety of other inclusion/exclusion criteria. No subjects were excluded on the basis of the DHI results.

Thus, 52 subjects were randomized to double-blind treatment with dalbavancin (39) or placebo (13). Fifty-one subjects completed the study, and one subject (multiple-dose dalbavancin) prematurely discontinued at the request of the subject.

**Threshold monitoring**

Using ASHA 1994 criteria for detection of ototoxic change, there was no evidence of ototoxicity in this study for any subject at any test interval for either the conventional or EHF ranges. Extreme change values for audiometry results for both ears combined by cohort are presented in Table 2.
The majority of subjects (20/21, 95% single-dose cohort; 17/18, 94% multiple-dose cohort; and 13/13, 100% placebo cohort) experienced an extreme change value no greater than ±10 dB. There were no significant changes in the conventional or high-frequency ranges at any time point for any of the 52 subjects. In addition, the DHI Score was zero for all subjects in all single- and multiple-dose groups at all assessment time points.

The results of the overall analysis indicated that any change over time was not different for frequency (treatment x frequency x time: F<1, df = 672, 1776). The power to detect this effect, if it had occurred, was >.99 when sphericity is assumed. If ototoxic change had occurred, any change would be expected to be different for frequency because ototoxicity usually first occurs in the high frequencies and then progresses to the lower frequencies. However, greater changes for the high frequencies did not occur in this data set even though the power to detect any difference for frequency was >.99.

There was no significant change over time interval that was related to treatment condition, (F<1, df = 56,148). The power to have detected this change, had it occurred, was .812. There was an overall change in threshold as a function of time interval (F = 2.268; df = 4, 148; p=.065). Thresholds improved by approximately 0.2 dB per test interval. This finding most likely reflects the subjects' acclimation to the testing procedure (test-retest at baseline = ± 5 dB). It should be noted that auditory threshold was assessed in 5 dB increments and that 0.2 dB does not even approach a significant change clinically. Further, an improvement in auditory threshold over time could not be secondary to ototoxic change.

The only other significant finding indicates that the frequency profiles of the different treatment groups (treatment x frequency) were not equivalent (F = 1.463; df = 168, 444; p = .001). This was likely attributable to the fact that the group frequency profile was affected by the small number of individuals in each treatment condition. However, these profiles did not change as a

Table 1. Subject Disposition

<table>
<thead>
<tr>
<th>Number (%) of subjects who:</th>
<th>n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Were screened</td>
<td>162 (100)</td>
</tr>
<tr>
<td>Failed auditory threshold criteria</td>
<td>48 (23)</td>
</tr>
<tr>
<td>Failed conventional frequency test</td>
<td>10 (6)</td>
</tr>
<tr>
<td>Did not attend hearing screening appt.</td>
<td>9 (.5)</td>
</tr>
<tr>
<td>Failed entry criteria other than hearing screen</td>
<td>27 (17)</td>
</tr>
<tr>
<td>Withdrew consent/unable to agree to schedule</td>
<td>11 (7)</td>
</tr>
<tr>
<td>Alternate subject screened but not dosed</td>
<td>15 (9)</td>
</tr>
<tr>
<td>Screened, enrolled, and dosed with study drug</td>
<td>52 (32)</td>
</tr>
</tbody>
</table>

Table 2. Subjects included in Various Dosing Protocols

<table>
<thead>
<tr>
<th>Number (%) of subjects who:</th>
<th>Single-Dose</th>
<th>Multiple-Dose</th>
<th>Placebo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrolled</td>
<td>21 (100)</td>
<td>18 (100)</td>
<td>13 (100)</td>
<td>52 (100)</td>
</tr>
<tr>
<td>Received any study drug</td>
<td>21 (100)</td>
<td>18 (100)</td>
<td>13 (100)</td>
<td>52 (100)</td>
</tr>
<tr>
<td>Completed study</td>
<td>21 (100)</td>
<td>17 (94)</td>
<td>13 (100)</td>
<td>51 (98)</td>
</tr>
<tr>
<td>Terminated before completion*</td>
<td>0</td>
<td>1 (6)</td>
<td>0</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

a. Subject did not complete study participation.
function of time (they were the same before and after study drug administration). The frequency profiles mean that, although all subjects had hearing thresholds within normal limits at baseline and throughout the course of the study, there was some intersubject variability in thresholds, although no subject showed significant change over time. Intersubject variability in thresholds, particularly for high-frequency audiometry, is well known even in normal subjects. Fortunately, this intersubject variability (i.e., differences between subjects’ auditory thresholds at baseline that persist over time) is not a major issue in ototoxicity monitoring, which monitors for changes over time within a given subject. Intrasubject variability for these measures was low and changes over time did not occur.

Only 3 subjects had any pre- to post-study drug administration changes in threshold that exceeded +10 dB, but those changes did not meet the ASHA criteria for ototoxic change. Two of those subjects were in the placebo groups and one subject was in the active group. None of the subjects had significant changes at adjacent frequencies or in both ears at a particular frequency. As previously mentioned, no subject met the ASHA 1994 criteria for ototoxic change at any test interval.

Also, no systematic relationship existed between dosing level and measured threshold. There was no evidence of increased thresholds at the time intervals tested, despite assumptions maximizing the possibility of finding such changes if they occurred.

Even for the subjects receiving the highest dalbavancin dosing levels of 1120 mg in the single-dose protocol and cumulative 1600 mg in the multiple-dose protocol, no subject demonstrated any evidence of ototoxic change in either hearing or balance. No subject met the criteria for ototoxic change or provided any subjective report of changes in auditory or vestibular function. Therefore, no false-positive or true-positive results were obtained for any subject in either the control or experimental groups for the auditory tests or DHI questionnaire. Even for the high-frequency audiometry testing above 8000 Hz, which is highly sensitive to even early ototoxic change, no significant change occurred in any subject at any time.

**DISCUSSION**

To our knowledge, this is the first report describing detailed audiologic methods to monitor for potential ototoxicity in a phase I clinical trial as part of the drug development process to be included in the submission of safety and efficacy data that is reviewed by the FDA before the drug can be marketed. The sponsor sought input in the design of the trial from the FDA prior to study implementation, and the study was conducted under GCPs, which are regulated by the FDA. These methods may serve as a reference for others conducting clinical trials including ototoxicity monitoring.

For auditory ototoxicity monitoring, several options are available. Certainly, testing in the conventional frequency range

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**Table 3. Threshold Results: Extreme Change Values (Worst Case Change in dB) for Both Ears Combined by Cohort**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Single dose n(%)</th>
<th>Multiple dose n(%)</th>
<th>Placebo n(%)</th>
<th>Total n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects Treated</td>
<td>21 (100)</td>
<td>18 (100)</td>
<td>13 (100)</td>
<td>52 (100)</td>
</tr>
<tr>
<td><strong>Extreme Positive Change</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 dB</td>
<td>14 (67)</td>
<td>11 (61)</td>
<td>7 (54)</td>
<td>32 (62)</td>
</tr>
<tr>
<td>10 dB</td>
<td>6 (29)</td>
<td>6 (33)</td>
<td>6 (46)</td>
<td>18 (35)</td>
</tr>
<tr>
<td>15 dB</td>
<td>1 (5)</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
</tr>
<tr>
<td><strong>Extreme Negative Change</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-5 dB</td>
<td>5 (24)</td>
<td>8 (44)</td>
<td>8 (62)</td>
<td>21 (40)</td>
</tr>
<tr>
<td>-10 dB</td>
<td>14 (67)</td>
<td>9 (50)</td>
<td>5 (38)</td>
<td>28 (54)</td>
</tr>
<tr>
<td>-15 dB</td>
<td>2 (10)</td>
<td>0</td>
<td>0</td>
<td>2 (4)</td>
</tr>
</tbody>
</table>
of 0.25 to 8 kHz for pure-tone thresholds is standard but can be insensitive to early ototoxic change. Both EHF audiometry in the 10 to 16 kHz range (Fausti et al, 1984a, 1984b; Rappaport et al, 1985; Kopelman et al, 1988) and otoacoustic emissions (Plinkert and Krober, 1991; Zorowka et al, 1993) can detect ototoxic change prior to changes in the conventional frequency range. However, significant change criteria to detect ototoxic change in otoacoustic emissions have not yet been developed. High-frequency audiometry has been well documented over the last few decades to detect ototoxic change early (Fausti et al, 1984a, 1984b; Rappaport et al, 1985; Kopelman et al, 1988). In the early years of investigation, intrasubject variability for EHF auditory thresholds was high but was largely caused by acoustic variances created when these highly directional waveforms were coupled to the ear. This transducer effect was the topic of numerous investigations and discussions (Fausti et al, 1979; Stelmachowicz et al, 1982; Tonndorf and Kurman, 1984; Northern and Ratkiewicz, 1985). Over the years, the problem was overcome by using carefully calibrated circumaural earphones (Fausti et al 1985; Frank, 1990, 2001) such as the Sennheiser HDA 200 we used in this study (Frank 2001).

When the EHF range is used for ototoxicity monitoring, it is important that intrasubject variability in this range not exceed the criteria for ototoxic change. Frank (2001) recently demonstrated that the intrasubject variability in the EHF range did not exceed that of the conventional frequency range in a group of normal-hearing listeners, nor did threshold variability exceed the ASHA criteria for ototoxic change using the transducer employed in this study. Using the ASHA 1994 criteria to determine significant ototoxic change in both the conventional and the EHF ranges in subjects exposed to a potential ototoxin has been reported in a previous study of aminoglycoside ototoxicity (Fausti et al, 1999).

Requiring threshold replicability within 5 dB at every frequency in each ear is a more stringent inclusion criterion than frequently recommended in other published studies of ototoxicity monitoring. This criterion was included to ensure that any threshold changes seen could not be attributed to simple poor subject reliability. Although this criterion did eliminate some potential study subjects, it also probably contributed to the absence of false-positive results among our subjects. However, this replicability criterion may be too stringent for clinical populations.

No guidelines exist for monitoring potential vestibulotoxicity in a clinical trial of a potential ototoxin. Most vestibular test protocols would be impractical for weekly monitoring of subjects. The DHI, although not designed for this purpose, worked well for this phase I study. Some of the questions of the DHI could possibly be eliminated or modified for vestibulotoxicity monitoring. However, using the DHI as a quick, noninvasive screen to determine if a subject needed further testing at some point seems appropriate.

There are also no guidelines specifically for monitoring potential glycopeptide ototoxicity. The ASHA 1994 threshold criteria for ototoxic change would seem to be appropriate for any ototoxicity monitoring; however, the monitoring schedule should be modified by the type of agent. If glycopeptides are ototoxic when delivered in the absence of other prior or concomitant ototoxins (e.g., aminoglycosides), the incidence appears to be extremely low. Consequently, in standard clinical practice, prospective monitoring of the patients receiving only a glycopeptide, with no prior or current exposure to other ototoxins, may not be warranted.

The absence of any evidence of ototoxicity in this new glycopeptide, dalbavancin, is not surprising. Glycopeptides, as a class, may not be ototoxic. If they are ototoxic, the incidence is very low and probably below 1 percent for vancomycin (Matzke et al, 1986; Brummett et al, 1989; Brummett, 1993; Garrelts et al, 1994; Cantu et al, 1994; Gendeh et al, 1998; Schaefer et al, 1999). It is possible that many of the cases of suspected glycopeptide ototoxicity in the literature were secondary to previous or concomitant aminoglycoside administration. Our exclusion criteria prevented aminoglycoside administration from being a factor in this phase I study. Naturally, the subject inclusion and exclusion criteria are critical in designing both sensitive and specific test procedures for clinical trials.
CONCLUSIONS

Even using these stringent audiologic criteria with highly sensitive audiologic testing, no dalbavancin ototoxicity, either auditory or vestibular, was observed for any dosing regimen for any subject in this dose-escalation phase I study. No significant change occurred, even for the very sensitive high-frequency audiometry measures through 16 kHz or for the DHI. These results suggest no evidence of dalbavancin ototoxicity even at the 1120 mg single dose and up to a total cumulative dose of 1600 mg administered over 1 week in the multible-dose cohorts. It is hoped that the audiologic procedures we used for this study will be helpful to future clinical trials evaluating the ototoxic potential of a new chemical entity or evaluating otoprotective agents.

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REFERENCES


