Insights Into Elevated Distortion Product Otoacoustic Emissions In Sickle Cell Disease:
Comparisons of Hydroxyurea-treated and Non-treated Young Children

Andrew Stuart and Sherri M. Jones
Department of Communication Sciences and Disorders
East Carolina University
Greenville, NC 27858, USA
Letitia J. Walker
Department of Communication Sciences and Disorders:
Missouri State University
Springfield, MO

Address reprint requests and corresponding author:
Andrew Stuart, Ph.D.
Department of Communication Sciences and Disorders
East Carolina University
Greenville, NC 27858-4353
252-328-9854
252-737-1111 (Fax)
sturta@mail.ecu.edu
Abstract

Distortion product otoacoustic emissions (DPOAEs) were examined in 15 normal-hearing African-American children between the ages of 6 and 14 years with homozygous sickle cell disease (SCD), who were on a regimen of hydroxyurea (HDU), a drug that reduces inflammatory processes and symptoms of SCD; a matched group of 15 African-American children with homozygous SCD not on HDU; and 15 African-American children with normal hemoglobin. DPOAEs were evoked by 13 primary tone pairs with $f_2$ frequencies ranging from 1000 to 4500 Hz. Increased DPOAE amplitudes, believed to be a precursor of eventual hearing loss, were evident in children with SCD who were not receiving HDU. Those taking HDU had DPOAE amplitudes similar to normal controls. These findings suggest that HDU, in addition to reducing symptoms of SCD, may play a role in inhibiting or preventing cochlear pathology and hearing loss in individuals with SCD.

Key Words: distortion product otoacoustic emissions; sickle cell disease; hydroxyurea

Abbreviations: ABR = auditory brainstem response; DPOAE = distortion product otoacoustic emission; HDU = hydroxyurea; HbSS = homozygous sickle cell disease; ICAM = intercellular adhesion molecule; $M$ = mean; OAE = otoacoustic emission; $p$ = probability; PECAM = platelet-endothelial cell adhesion molecule SCD = sickle cell disease; $SD$ = standard deviation of the mean; SOAE = spontaneous otoacoustic emission; TEOAE = transient evoked otoacoustic emission; VCAM = vascular cell adhesion molecule.
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The hallmarks of sickle cell disease (SCD) are hemolytic anemia and vaso-occlusion as a result of intracellular polymerization of deoxyhemoglobin S. Occlusion occurs from the level of the microvasculature to large arteries. The precursor of vaso-occlusion is activation of inflammatory processes by erythrocyte, and to a lesser extent leucocyte, interactions with the postcapillary venule endothelium. The obstruction or trapping of sickle cells is secondary to adhesion of erythrocytes on the venous side of microcirculation. The sequela includes local hypoxia, increased erythrocyte sickling, and spread of inflammation to adjacent tissue (Elion et al., 2004; Stuart and Nagel, 2004).

Given the microvasculature of the cochlea (Haupt et al., 1993; Scheibe et al., 1997; Slepecky, 1996), one would suspect that cochlear function would be particularly vulnerable in those with SCD. Indeed, histopathological changes in the temporal bone and degenerative changes in the organ of Corti consonant with hypoxia have been observed with SCD (Morgenstein and Mance, 1969). Also consistent with this speculation is the observation of a higher prevalence of sensorineural hearing loss among those with SCD (Adams and Benson, 1992; Ajulo et al., 1993; Ashoor and Al-Awamy, 1985; Atsina and Ankra-Badu, 1988; Crawford et al., 1991; Forman-Franco et al., 1982; Friedman, et al., 1980; Piltcher et al., 2000; Todd et al., 1973). Hearing loss has a progressive nature in SCD as the prevalence is lower in children than adults (Gentry et al., 1997; Koussi et al., 2001; MacDonald et al., 1999; Odetoynbo and Adekile, 1987).
and the degree becomes more advanced with increasing age (Onakoya et al., 2002; Piltcher et al., 2000).

It is generally recognized that evoked otoacoustic emissions (OAEs) are more sensitive in revealing early or sub-clinical cochlear damage versus standard behavioral testing (e.g., Lonsbury-Martin et al., 1993; Prieve et al., 1997; Schweinfurth et al., 1997; Shera, 2004). OAEs reflect nonlinear distortion and linear reflection mechanisms in the cochlea generated by outer hair cell activity in response to acoustic stimulation (Shera, 2004). Energy from this activity is partly released back through the middle ear to the ear canal reappearing as sound. Distortion product otoacoustic emissions (DPOAEs) are intermodulation products produced by the cochlea when presented with two closely spaced simultaneous pure tones (i.e., $f_1$ and $f_2$). DPOAE amplitude increases with increasing input levels until saturation and varies as a function of stimulus frequency. Following cochlear insult (e.g., hypoxia, noise, or ototoxic drugs), DPOAEs are reduced in amplitude or are absent (Kemp, 2002).

We originally investigated DPOAEs in normal-hearing African American school-aged children with SCD in an effort to see if sub-clinical cochlear damage was present (Downs et al., 2000; Walker et al., 2004). Paradoxically, an increase in DPOAE amplitude was observed and the likelihood of detecting a DPOAE response was not related to the disease status or middle ear function. That is, Downs et al. (2000) reported that normal-hearing children with SCD had larger DPOAE amplitudes than children with normal hemoglobin and that the prevalence of DPOAEs did not differ between the two groups. Considering that the integrity of the middle ear system directly influences OAE
characteristics, Walker et al. (2004) undertook a concurrent investigation of DPOAEs and outer/middle ear function with tympanometry. DPOAE amplitudes were again significantly larger for children with SCD, but there were no group differences in any of the middle ear indices (i.e., peak compensated static acoustic admittance, tympanometric width, tympanometric peak pressure, ear canal volume, and middle ear resonance frequency). Their findings were consistent with the notion that increased DPOAE amplitudes could not be attributed to differences in outer/middle ear function as assessed with tympanometry.

DPOAE amplitudes have been reported to increase before reduction or loss secondary to localized cochlear lesions in animal studies suggesting that increased DPOAE amplitudes may precede the manifestation of measurable behavioral changes in cochlear pathology (Huang et al., 2005; Kakigi et al., 1998; Raveh et al., 1998). It stands to reason that increased DPOAE amplitude may be related to the inflammatory process of SCD since markers of vascular inflammation have been observed in the cochlea following other cochlear lesions (e.g., Suzuki and Harris, 1995; Zhang et al., 2000) and inflammatory responses can lead to cochlear damage and hearing loss (Ryan et al., 2002).

Hydroxyurea (HDU) is currently the only known drug that reduces the frequency of vaso-occlusive crisis, pain, and transfusion needs in those suffering from SCD (Halsey and Roberts, 2003; Stuart and Nagel, 2004). HDU was originally investigated for its ability to increase fetal hemoglobin with a concomitant decrease in sickle cell hemoglobin, as it was known that patients with the elevated fetal hemoglobin experience milder forms of SCD. Since clinical improvement was noticed in patients prior to
significant increase in fetal hemoglobin, other properties of HDU had to mediate recovery. It is now believed that HDU, in addition to increasing fetal hemoglobin, reduces erythrocyte and leucocyte endothelial adhesion thereby reducing inflammatory processes; enhances erythrocyte rheology and myelosupression; and facilitates vasodilatation by increased nitric oxide release (Halsey and Roberts, 2003).

We subsequently hypothesized that if HDU reduces the inflammatory process, one should observe a difference in DPOAE responses in those with SCD receiving HDU versus those who are not. Toward that end, we examined DPOAEs in normal-hearing African-American children with homozygous (HbSS) SCD who were on a regimen of HDU; a matched group of African-American children with homozygous SCD not on HDU; and African-American children with normal hemoglobin.

Method

Participants

Forty-five normal-hearing, African-American children between the ages of 6 and 14 years participated. Fifteen African-American children ($M = 10.5$ years, $SD = 2.9$; nine males, six females) with HbSS SCD who were on a regimen of HDU and a matched group of 15 African-American children ($M = 9.1$ years, $SD = 1.8$; six males, nine females) with HbSS SCD not on HDU were selected from the East Carolina University School of Medicine Sickle Cell Clinic at Pitt County Memorial Hospital, Greenville, NC. The average HDU regimen length was 27.3 months ($SD = 25.7$, range 3-77 months). HDU was administered 15 mg/kg orally once a day. The average HDU dosage was 805 mg ($SD = 351$, range 286-1500).
All of the children with SCD were asymptomatic (i.e., not in crisis) at the time of testing. A control group of 15 African-American children \((M = 9.9\) years, \(SD = 2.9\); six males, nine females) with normal hemoglobin also participated. There was no significant difference between the mean ages of the three groups of participants \((p = .32)\). All participants presented with normal-hearing sensitivity defined as having pure-tone thresholds at octave frequencies from 500 to 4000 Hz of \(\leq 20\) dB HL (American National Standards Institute, 1996). There was no significant difference between the mean pure tone thresholds between groups \((p = .77)\). Participants also presented with normal otoscopy and negative histories of otitis media (i.e., according to parental report). Normal otoscopy was defined as an absence of ear drainage, a previously undetected structural deficit, ear canal abnormalities (i.e., obstruction, impacted cerumen, foreign object, blood or secretion, stenois or atresia), otitis externa, a perforated tympanic membrane or other abnormality of the tympanic membrane (American Speech-Language-Hearing Association Panel on Audiologic Assessment, 1997).

**Apparatus**

DPOAEs at 2f1-f2 were collected with a Grason-Stadler GSI-60 DPOAE System (Revision 4.2.0) interfaced with a personal computer (Compaq Model Deskpro 2000). The protocol for collection of DPOAEs was identical to that of Downs et al. (2000) and Walker et al. (2004). An \(f_2/f_1\) ratio of 1.22 was used with the primary tones to evoke DPOAEs. Recordings were obtained at \(f_2\) frequencies of 1078, 1218, 1359, 1546, 1734, 1921, 2156, 2437, 2718, 3093, 3468, 3890, and 4359 Hz. These frequencies were
selected because DPOAE test performance is best in this mid to high \( f_2 \) frequency range (Gaskill and Brown, 1990; Gorga et al., 1993a,b; Kimberly et al., 1997).

Primary tones were presented at two levels. Primary \( L_1 \) and \( L_2 \) levels were 70 and 60 dB SPL and 50 and 40 dB SPL for high and low levels, respectively. A sequential signal presentation was utilized. Time domain averaging for DPOAE data collection was employed. Ten averages were acquired for each data point. A 24,000 Hz sampling rate was used for all conditions. Frame rejection ensued if \( L_1 \) and \( L_2 \) were out of tolerance by \( \pm 5 \) dB and/or ambient noise levels exceeded 30 dB SPL. DPOAE collection ended when either of the following occurred: test time exceeded 32 seconds or 1500 frames; 50 occurrences of frame rejection due to excessive ambient noise; and/or 20 occurrences of frame rejection due to \( L_1 \) and \( L_2 \) being out of tolerance for at least 20 frames. The test was accepted when either of the following occurred: 10 frames were averaged; the average noise level was less than -6 dB SPL; and/or either the DPOAE was 3 dB above the noise floor or the absolute noise level was less than -12 dB SPL.

Procedure

The University and Medical Center Institutional Review Board at East Carolina University reviewed and approved the research study prior to any data collection. Control participants were tested in a double wall sound-treated audiometric suite (Industrial Acoustics Corporation) at the Department of Communication Sciences and Disorders at East Carolina University. All children with SCD were tested in a quiet room at Pitt County Memorial Hospital. Both rooms met specifications for permissible ambient noise
DPOAEs were obtained for one randomly selected ear for all participants. For collection of the DPOAEs, participants sat upright while a probe tip (GSI 1700-9660) was positioned securely in the ear canal such that the proximal edge of the probe tip flange was flush with the entrance to the external auditory meatus. DPOAEs were estimated as the amplitude in the frequency bin for the cubic distortion product 2f1-f2. The average amplitude of the three frequency bins on either side of the cubic distortion product bin served to estimate the noise floor (Gorga et al., 1997). The noise floor was set at -6 dB SPL. A DPOAE was deemed to be present if response amplitude was 3 dB larger than the noise floor. In few cases where participants did not sit quiet, two or three DPOAE measures were obtained. In these cases, all repeated measures were averaged. In cases where data points were missing (i.e., 4%), they were replaced by the mean of two valid surrounding values (i.e., the two data points in each adjacent frequency bin).

Results

The mean DPOAE amplitudes as a function of group, presentation level, and f2 frequency are displayed in Figure 1. A four factor mixed analysis of variance was undertaken to investigate differences in mean DPOAE amplitudes as a function of group, gender, primary tone level, and f2 frequency. The results of this analysis are presented in Table 1. As evident in Table 1, significant main effects of group, presentation level, and f2 frequency and significant interactions of frequency by group and frequency by level (p < .05) were found.
Discussion

The effects of presentation level and $f_2$ frequency on DPOAE amplitudes observed across all three groups of children are well accepted (Kemp, 2002; Shera, 2004). DPOAE amplitudes of the children in the control group are similar with previously reported data on normal-hearing school-aged children (O'Rourke et al., 2002; Owens et al., 1993; Prieve et al., 1997). The group effect and frequency by group interaction supports our hypothesis that HDU reduces the inflammatory process whereby DPOAE amplitudes in children on HDU were similar to those of the normal controls. It is difficult to conclude that the disruption of the terminal blood supply to the cochlea is responsible for larger DPOAEs observed in these children with SCD as acute hypoxia and ischemia have been shown to reduce DPOAE amplitudes (Koga et al., 2003; Mom et al., 1999; Schweinfurth and Cacace, 2000; Telischi et al., 1999). It may be the case that children with SCD not on HDU may have more spontaneous otoacoustic emissions (SOAEs) since it is known that SOAEs enhance DPOAE amplitude in the frequency region of the SOAE (e.g., Lonsbury-Martin et al., 1990; Prieve et al., 1997). Unfortunately SOAEs were not assessed in this cohort of children. It is unlikely, however, that this is the cause of group differences in DPOAE amplitudes as there is no evidence to suggest that only those children with SCD and not on HDU would exhibit more SOAEs. Further, it is unlikely that they would have multiple SOAEs to the extent that larger DPOAE amplitudes would be seen at 10 $f_2$ frequencies. When multiple SOAEs are evident there are typically on average four SOAEs (Lonsbury-Martin et al., 1990; Talmadge et al., 1993). We offer
another possibility namely the sequela of vaso-occlusive events and their impact on cochlear function. Our reasoning is as follows:

First, more contemporary research findings support the notion that SCD be viewed as a chronic inflammatory disease. For example, Belcher et al. (2003) measured several markers of vascular inflammation (i.e., vascular cell adhesion molecule [VCAM], intercellular adhesion molecule [ICAM], platelet-endothelial cell adhesion molecule [PECAM]) and demonstrated that all were significantly elevated in mice with transgenic SCD. Other evidence for inflammatory markers and processes has also been described (see Stuart and Hagel, 2004 for a review). Active vascular inflammation appears to be present in SCD even in an asymptomatic state (Chies and Nardi, 2001). In fact, exposure to sickle blood cells in vitro activates vascular endothelium inducing cell adhesion molecules (Brown et al., 2001). In addition, Kaul and Hebbel (2000) showed that a brief episode of hypoxia followed by reoxygenation actually triggered inflammatory processes (e.g., leukocyte-endothelium interaction and cell adhesion molecules) in SCD, processes that were not activated in normal mice post-hypoxia.

If SCD is a chronic inflammatory disease, is there evidence that the latter could exist in the cochlea and lead to hearing loss? The answer is yes: Suzuki and Harris (1995) investigated the presence of ICAM-1 within the inner ear following induction of labyrinthitis. They detected the expression of ICAM-1 within the spiral modiolar vein and collecting venules of the cochlea suggesting that ICAM-1 expression may play a role in inner ear inflammation. Zhang et al. (2000) demonstrated VCAM-1 expression on the endothelial surface of the spiral modiolar vein and collecting venules in induced
labyrinthitis. VCAM-1 expression persisted for up to two weeks. Zhang et al. suggested that VCAM-1 is linked to infiltration of inflammatory cells into the cochlea. Such an inflammatory response can lead to cochlear damage and hearing loss (for a review see Ryan et al., 2002).

Can inflammatory processes and/or other cochlear insults generate increased DPOAE amplitudes? As noted above, increased OAE amplitudes have been observed in animals following localized cochlear lesions. Raveh et al. (1998) examined transient evoked otoacoustic emissions (TEOAEs) and DPOAE amplitudes before and after local thermoprobe lesioning in the apical and middle turns of the cochlea of 11 adult chinchillas. Their most interesting observation was increased OAE amplitudes at frequencies basal to the lesioned frequency areas. Kakigi et al. (1998) measured hearing status in chinchillas with DPOAEs and TEOAEs before, during, and after amikacin treatment. Kakigi et al. (1998) found that when the basal most hair cells of the cochlea were damaged, OAE amplitudes increased particularly for stimulus frequencies bordering the damaged tonotopic region. OAEs decreased when hair cell damage was more extensive. They suggested that increased OAE amplitudes might precede the expression of hearing loss. Finally, Huang et al. (2005) reported a paradoxical enhancement in DPOAE amplitude in guinea pigs following salicylate injection. Following a single injection, DPOAE amplitude decreased but following a 14-day course of injection DPOAE amplitude progressively increased to a significant level relative to baseline. Four weeks following cessation of salicylate injection, the DPOAE amplitude increase
reversed to the normal baseline level. The normal control specimens showed no change in DPOAE amplitude over the same period.

Increased OAE amplitudes have also been observed in humans. Zorowka et al. (1993) reported increased TEOAE amplitudes in newborns with perinatal infections that were receiving aminoglycosides. Seventeen infants were tested shortly after birth following the first and before receiving a second dose of the antibiotic ampicillin plus the aminoglycoside tobramycin. Within 24 hours of their last aminoglycoside dose, a second TEOAE measurement was obtained. Five to 10 days later at retest, 10 of 21 ears demonstrated an increase in TEOAE amplitude while nine ears displayed no change and two ears a decrease in TEOAE amplitude relative to the initial test. Cevette et al. (2000) also reported increased DPOAE amplitudes following ototoxic medication. In two case reports, hearing thresholds and DPOAEs were serially recorded in a 30 year-old male and a 64 year-old female receiving cisplatin. Measures were obtained at baseline prior to administration of cisplatin and a one, two, four, and six months post administration. Both individuals showed significant bilateral increases in DPOAE amplitudes at one month bilaterally followed by a significant decrease in one case or an absence in case of DPOAEs in subsequent tests.

The mechanism underlying increased DPOAE amplitude seen in children with SCD remains to be elucidated. It is possible that inflammatory process(es) somehow affect the mechanics of the basilar membrane and/or organ of Corti. Kakigi et al. (1998) suggested that the site of lesion basal to the area being monitored might be responsible whereby the increased OAE amplitude is a “result of a more effective basal transmission
signal across a less active, and therefore less interfering cochlear region” (p. 371). This speculation is not consistent with the significant interaction of frequency by group; namely that both groups of children with SCD showed similar increased DPOAE amplitudes in the lower frequencies (i.e., $f_2 < 1546$ Hz). Here the elevated amplitudes in the low frequencies must be interpreted as impairment of the apical region of the cochlea in the case of the children receiving HDU. This is not consistent with the common observation that loss in function typically follows a basal to apical pattern and recovery generally happens at the distal margin of the insult (e.g., Nicol et al., 1992). The mechanisms behind the differences and similarities, between children with SCD treated and not treated with HDU, remain to be elucidated.

It may also be the case that there is some dysfunction or reduction in the efferent suppression of outer hair cell activity that is responsible for increased DPOAE amplitude seen in children with SCD. This could be a consequence of aberrant medial olivocochlear neuron function or a disruption of olivocochlear efferent transmitter function. Some pharmacological agents are known to disinhibit the OAE response (e.g., Chen et al., 1998; Drexl et al., 2004; Kujawa et al., 1994). There is also evidence of enhancement of OAEs following drug administration or insult to the olivocochlear efferent system in some individuals (Berlin et al., 1993, 1994). Interestingly, if elevated OAEs prove to be a consequence of aberrant medial olivocochlear efferent system function then SCD may share some underlying mechanisms seen with age-related functional decline of the medial olivocochlear efferent system see in humans and animals. In fact, functional decline in the medial olivocochlear efferent system precedes hair cell degeneration (Jacobson et al.,
2003; Kim et al., 2002). However, this seems unlikely as there is no evidence to suggest that HDU affects the central efferent system and therefore mediates recovery of outer hair cell function via the medial olivocochlear efferent system. Finally, Kemp (1986) has suggested an “adaptive mechanism” that allows the cochlea to return to a normal state following insult. His finding of OAE amplitude rebounding to normal levels following noise exposure has also been evidenced following exposure to ototoxic treatment (Whitehead et al., 1992).

If one views SCD as an inflammatory process and increased DPOAE amplitudes as a consequence of the process, what then is the role of HDU? As noted above, HDU diminishes the inflammatory processes in SCD (Halsey and Roberts, 2003; Stuart and Nagel, 2004). It stands to reason then that the observed differences in DPOAE amplitude between the two groups of children with SCD in this study be viewed as a drug effect. The mechanism of HDU in reducing vaso-occlusive events in SCD has been suggested as decreased ICAM (Conran et al., 2004) and VCAM levels (Saleh et al., 1999, 1998), reduced adhesion molecule expression in leucocytes (Okpala et al., 2002), decreased vaso-constrictor peptide through down regulation of gene expression (Brun et al., 2003), increased vasomotor tone via intravascular and intraerythrocytic generation of nitrous oxide (Galdwin et al., 2002), a decrease in polymorphonuclear neutrophil adhesion to endothelium (Benkerrou et al., 2002), decrease in adhesion receptor expression (Styles et al., 1997), and decreased erythrocytic adhesion to protein thrombospondin and laminin (Hillery et al., 2000). If HDU diminishes the inflammatory processes then the rebound of
the DPOAE amplitudes toward a normal level reflects a reduction in the mechanism(s) of the yet undetermined cause of the increased amplitude.

These findings suggest that not only does HDU prevent SCD crisis but it also may play a role in minimizing abnormal cochlear function and/or the restoration of normal function as revealed by DPOAEs. That being the case, HDU may prevent or delay hearing loss in those with SCD. As the long-term efficacy of HDU treatment in children with SCD is examined, beneficial or detrimental effects of HDU on hearing function should also be examined. It may be the case, in those with SCD who undergo a long-term regime of HDU, that a lower prevalence and less progression in extent and degree of sensorineural hearing loss with increasing age be evidenced. Finally, if the increase in OAE amplitudes precedes the expression of detectable cochlear pathology, it remains to be determined at what point normal hearing children who are not on a regime of HDU begin to display a reduction in amplitude and a loss in OAE expression that eventually accompanies demonstrable decreases in behavioural hearing sensitivity that accompanies SCD. Recall from above that hearing loss is progressive in nature with SCD as the prevalence is lower in children than adults, yet the severity of hearing loss becomes greater with increasing age.
References


Table 1

*Summary Table for the Three-Factor Mixed ANOVA Investigating Mean DPOAE Amplitude as a Function of Group, Gender, Primary Tone Level, and $f_2$ Frequency.*

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*Note.* *$p < .05$; repeated measures factor $p$ values following a Huynh-Feldt correction (Huynh and Feldt, 1976). Effect size and power at $\alpha$ of .05 are indexed by $\eta^2$ and $\phi$, respectively.*
Figure Caption

Figure 1. Mean DPOAE amplitude as a function of group, $f_2$ frequency, and stimulus level.