

# The Effects of Furosemide and Hypoxia on Click-Evoked Otoacoustic Emissions in Anesthetized Guinea Pigs†

Sun O Chang and Ha Won Jung

Department of Otolaryngology, Seoul National University, College of Medicine, Seoul 110-744, Korea

**= Abstract =** From the concepts of well known ototoxic effects of loop diuretics and hypoxic state, twenty anesthetized guinea pigs with diuretics and/or hypoxia were evaluated with click-evoked otoacoustic emissions (CEOAEs) by time-sequential monitoring. Furosemide 25 mg/kg was infused intravenously and CEOAEs were measured before and 5, 10, 20, 30, 45 and 60 minutes after injection in 8 guinea pigs. Both echo responses and reproducibilities were decreased significantly at 10 minutes after injection and showed minimal level at 20 minutes. Recovery of CEOAEs was noted by spectral analysis at 30 minutes after injection, and full recovery of CEOAEs was evident after then. At 60 minutes after injection, hypoxia of 20 seconds by turning off the artificial respirator was added. The CEOAEs showed statistically significant decreases compared with those of prehypoxic state ( $p < 0.01$ ). The CEOAEs of 8 guinea pigs with injection of furosemide 50 mg/kg showed decreased responses and no recoveries for the 60 minutes observation periods. Findings from this study support the clinical utility of CEOAEs as a screening test for early detection of various ototoxicities.

Key Words: *Furosemide, Hypoxia, Click, Evoked otoacoustic emission, Guinea pig*

## INTRODUCTION

The evoked otoacoustic emission (EOAE) is a reflection of the sound waves to a specific acoustic stimulus. It is generated from the cochlea and emitted via ossicles and the tympanic membrane retrogradely. In 1978, Kemp reported the presence of faint reflections

of sound waves that were measured by a microphone inserted into the external ear. The presence of the EOAE provides evidence of a functioning cochlea (Collet 1978; Stevens 1988). Thus it can be used as a functional cochleogram that represents the physiology of the peripheral sound processing mechanism (Kemp *et al.* 1986).

The EOAE test can be performed without anesthesia in human subjects. What should be considered first on measuring the click evoked otoacoustic emission (CEOAE) is adequate fitting of the probe in the external ear canal. Additional changes in acoustic damping

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서울대학교 의과대학 이비인후과학교실: 장선오, 정하원

(electric impedance) of earprobe cause different characteristics of emissions (Zwicker 1990).

Measuring of CEOAEs in guinea pigs was thought to be a difficult problem because of their short latency and duration. The authors' data on the profiles of the CEOAE findings in normal guinea pigs(1992) showed stable findings for test-retest reliability, even though the thresholds were higher than human ones. And the predominant response was shifted to higher frequencies according to the spectral analyses.

Loop diuretics cause interstitial edema of stria vascularis and secondary hair cell changes. These changes are reversible and occur in high dose intoxication (Quick and Duvall 1970; Kohonen and Janhidinen 1970). EOAEs could be involved with reduction or elimination of its amplitude (Anderson and Kemp 1979; Anderson 1980; Kemp and Brown 1984; Wilson and Evans 1983). Hypoxia also reduces the amplitude of OAE in laboratory animals (Kim 1980; Schmidt and Adams 1981; Lonsbury-Martin *et al.* 1987). Whether the ingestion of loop diuretics and the application of hypoxia have synergistic effects on the reduction of OAEs is not clear.

This study was planned to evaluate the influence of furosemide and superimposed hypoxia on the profiles of the CEOAE. The hypoxic state was artificially created for evaluation of a synergistic insult to inner ear having altered physiology by furosemide infusion.

The primary intention of this study was to elucidate the efficacy of the CEOAE test as a surveillance index for detecting the effects of ototoxic drugs and toxic environments.

## MATERIALS AND METHODS

### Selection and manipulation of test animals

Twenty albino guinea pigs weighing 250 to 400 gm and having normal Preyer reflexes were used. The tympanic membrane was assessed to be normal under microscopic examination. Any

animal which failed to show CEOAEs in a screening test was excluded from this study.

Anesthesia was initiated with an intraperitoneal injection of sodium pentobarbital (Nembutal) 30 mg/kg. The animal was tracheotomized in supine position. After intramuscular injection of Myoblock (1.25 mg/kg), respiration was controlled with an artificial ventilator (Narishige) at the rate of 60 times/minute and 6-8 ml of tidal volume. Body temperature was maintained by convection heat from the surgical light. The total time of the experiment did not exceed 2 hours, so booster injections of the anesthetic agent were not necessary. After the experiment, the animal was sacrificed immediately.

### Measurement of the CEOAE

This experiment was conducted using the ILO88 Otodynamic Analyzer developed by Bray and Kemp (1987). The click stimuli elicited by 80  $\mu$ sec electrical pulses were used with a frequency range of 0-6 kHz and an interstimulus repetition rate of 20 msec. Figure 1 shows the system block diagram used in this study.

The infant-style ear probe containing an earphone and a microphone was connected to the amplifier and the averaging computer system. Tight fitting of the probe on the EAC is a very important step on measuring the CEOAE (Zwicker 1990). We performed this experiment in a noise shielded booth with the animal deeply anesthetized to minimize external and internal noise. The artificial ventilator was also placed out of the booth to minimize noise.

Threshold for the noise level was set on the

### ILO 88 Otodynamic analyser hardware configuration

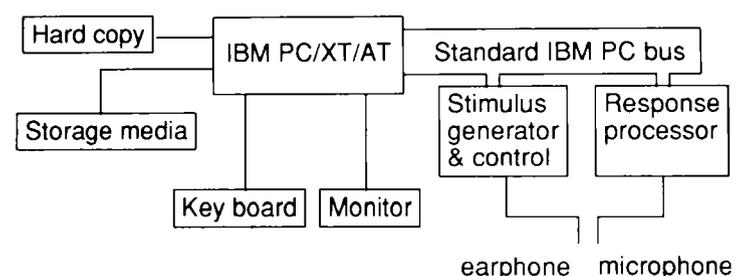


Fig. 1. System block diagram used in this study

IBM PC and the CEOAE was measured using the standard setting of the ILO88 (260 repetition, window from 2.5 msec to 20 msec). The amplitude of echo response above 5 dB and the reproducibility above 50% were regarded as positive responses (Kemp 1990).

### Injection of furosemide and induction of hypoxia

After the preparation of the animal, a small probe was fitted into the external ear canal of the guinea pig. Measurement of the CEOAE was started from 0 dB test gain (84 dB peak SPL). Sequential measurement of the CEOAE was done by ascending and descending the test gain by 5 dB steps and eventually determining the minimal threshold of the click that suffices the reference value of a positive CEOAE. Variable parameters manifested on the CEOAE at a given threshold level were compared with those in a different ototoxic environmental setting. The animals were divided into four groups (Table 1);

First group: Respiration of four guinea pigs was halted by turning off the respirator for 100 seconds and the CEOAE was measured immediately after the cessation of artificial respiration.

Second group: The left internal jugular vein of eight guinea pigs was exposed surgically and 25 mg/kg of furosemide was infused. The CEOAE was checked 5, 10, 20, 30, 45, and 60 minutes after the infusion of furosemide. These guinea pigs were exposed to 20 seconds of additional hypoxia, sixty minutes after the infusion of furosemide, and the CEOAE was checked again.

Third group: Furosemide at a dosage of 50 mg/kg was infused into the eight guinea pigs as

in the second group. The CEOAE was checked sequentially 5, 10, 20, 30, 45, and 60 minutes after the infusion.

### Statistical analysis

Statistical analysis was performed by the SAS program. The clinical significance of the experiment was tested with the Wilcoxon signed rank test within p value of 0.01.

## RESULTS

### The CEOAE findings under the hypoxic environment

The threshold level of the CEOAE was checked in the first group (four guinea pigs). After identifying the threshold, the artificial ventilator was disconnected for 100 seconds and the CEOAE at the preset threshold level was checked right after the hypoxic event.

The hypoxic state for 100 seconds proved to have no statistically significant impact on the profiles of the CEOAE. One example is displayed in Figure 2. Both amplitude of echo response and reproducibility did not show any significant change.

### The CEOAE findings after exposure to furosemide.

The amplitude of echo responses and reproducibility of the second group (eight guinea pigs) which were infused with 25 mg/kg furosemide showed decreased parameters from 10 minutes to 45 minutes ( $p < 0.01$ ). The amplitude and the reproducibility of the CEOAE were decreased from 10 minutes after infusion, to their lowest value at 20 minutes after infusion, and recovered to pre-infusion levels by 45 and 60 minutes after infusion (Fig. 3). Panel A in Fig.

Table 1. Groups of experimental animals (N=20 guinea pigs)

Group	No. of animals	Dosage of furosemide	Hypoxia exposure
I	4	(-)	100 sec
II	8	25 mg/kg	20 sec
III	8	50 mg/kg	(-)

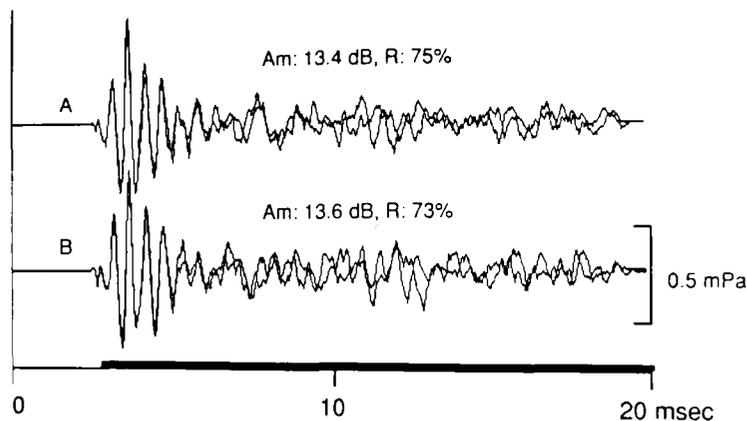


Fig. 2. Time domain responses of CEOAEs to 20 dB test gain (104 dB pSPL) click stimuli before (A) and after (B) the hypoxic exposure for 100 seconds in a guinea pig. Am: Amplitude, R: Reproducibility

3 shows the changes in the amplitude of echo response of 8 guinea pigs and panel B shows the changes of the reproducibility. Panel C plots the changes in both measures, with the horizontal plane indicating the amplitude of echo response and the vertical plane indicating the reproducibility.

The echo response and the reproducibility in the CEOAE of the 8 guinea pigs which were infused with 50 mg/kg furosemide (the third group) showed statistically significant decrease right after the exposure. There was no evidence of recovery of the echo response and the reproducibility after 60 minutes had passed ( $p < 0.01$ ). Fig. 4 depicts the CEOAE findings of

the third group.

### Additive effect of hypoxia on the furosemide infusion

The variables in the CEOAE were normal at 60 minutes after the exposure to the furosemide at 25 mg/kg. Additional exposure to the hypoxia for 20 seconds after recovery from the furosemide infusion resulted in dysmotility of cardiac contraction during the hypoxic event. Profiles of the CEOAE immediately after the combined exposure revealed a statistically significant additive decrease in the echo response and the reproducibility than those caused by the separate exposures ( $p < 0.01$ ). Figure 4 displays the results of the additive insult of hypoxia in animals receiving furosemide (25 mg/kg).

## DISCUSSION

In this study, a deep anesthesia was maintained with the artificial ventilator via the tracheotomy tube to minimize noise from the test animal. Deep anesthesia was also necessary to maintain access with the intravenous drug-administration route.

We used the small sized probe tip commercialized by Otodynamics Ltd. This probe tip had the merit of superior detection capability of high frequency emission above 3 kHz and

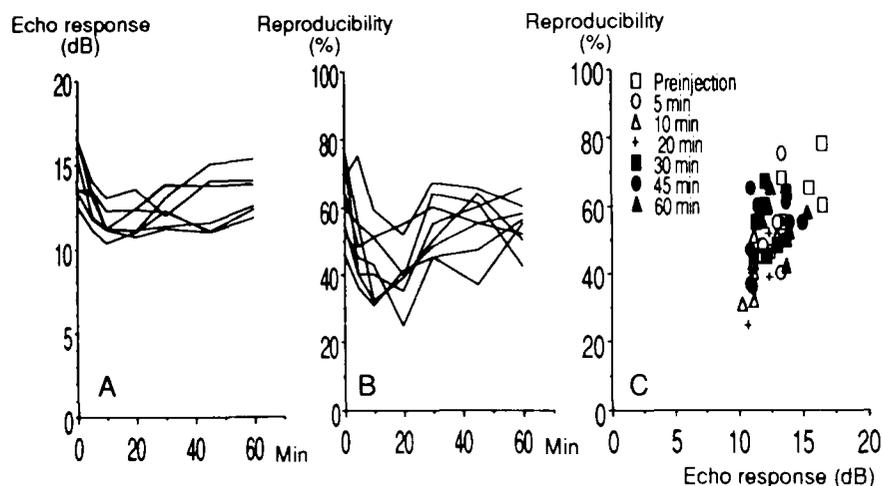


Fig. 3. Click evoked otoacoustic emissions in furosemide 25 mg/kg group (8 guinea pigs) to the given threshold level of click stimuli. Panel A: time domain changes of echo amplitude, B: time domain changes of reproducibility, C: amplitude and reproducibility

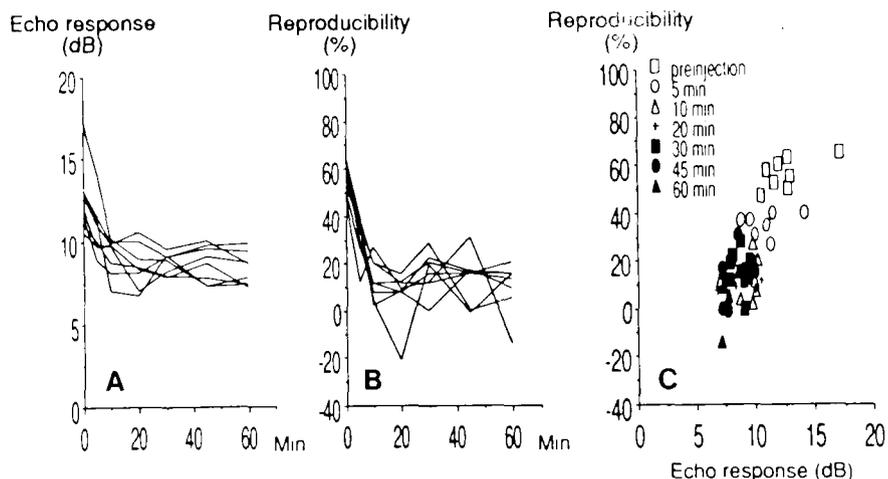


Fig. 4. Click evoked otoacoustic emissions in furosemide 50 mg/kg group (8 guinea pigs) to the given threshold level of click stimuli. Panel A: time domain changes of echo amplitude, B: time domain changes of reproducibility, C: amplitude and reproducibility.

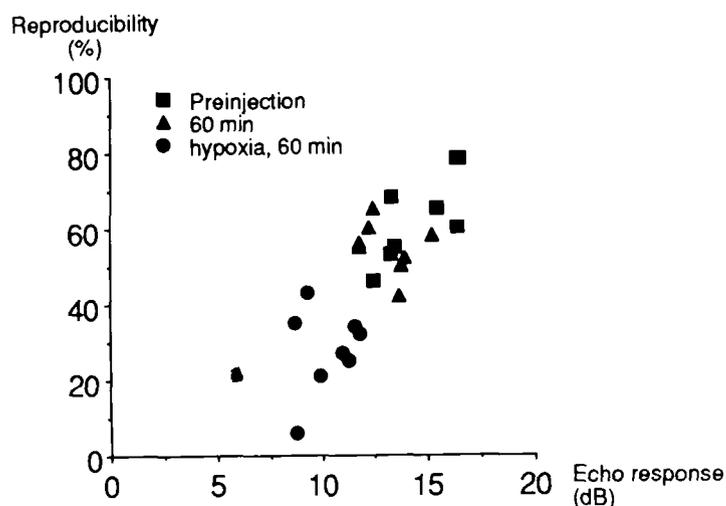


Fig. 5. Click evoked otoacoustic emissions in furosemide 25 mg/kg group with hypoxia for 20 seconds at the end of 60 minutes' observation period (8 guinea pigs, same group as Figure 3) to the given threshold level of click stimuli.

capability of eliminating the distortion of the response by the stimulus itself (Kemp *et al.* 1986). The fitting procedure must not be so complete as that of the tympanometry test, but the noise should be restricted to an adequate level for a significant test. In this study, malleable silicon rubber sealing was applied around the tip to promote the correct fitting to the ear canal. The tip of the probe must be directed to the tympanic membrane without being blunted against the canal walls (Kemp 1986; Kim 1980; Bonfils and Uziel 1989; Bonfils *et al.* 1989).

The profiles of the CEOAE vary with different

subjects, but they have homogenous features for the same subjects that were tested at different times under the same stimulus intensity (Kemp *et al.* 1986). Positive echo responses were regarded as functioning outer hair cells, especially within middle frequency region (Collet *et al.* 1989).

The CEOAE has a benefit of early detection capability of cochlear lesion, e.g any disease process, change due to exposure to noise, and exposure to ototoxic drugs. Temporary threshold shift after exposure to noise has been recorded objectively with the CEOAE in human subjects (Zurek 1985; Kemp *et al.* 1989). With ototoxic drugs, the initial decrease and the gradual recovery of the echo response of the CEOAE have variable time intervals according to the kind of drug, that had already been previously observed in patas monkeys in which loop diuretics had been infused (Zurek 1985; Kemp *et al.* 1989).

The ototoxic effect of furosemide and hypoxia had been a proven cochlear manipulation method by many authors, but the effects on CEOAEs were not common. So these were selected as the test materials for evaluation of the ototoxic effect manifested in the changes of the CEOAE.

Hypoxic state made with cessation of respiration for a period of 100 seconds in 4 guinea pigs had no statistically significant

influence on the CEOAE.

For the guinea pigs that were infused with 50 mg/kg of furosemide, the variable parameters of the CEOAE were decreased immediately after the infusion and there was no evidence of recovery even after 60 minutes had passed. The other group exposed to furosemide at 25 mg/kg concentration had decreased parameters of the CEOAE and the lowest value at 20 minutes after the infusion. They began to recover from 30 minutes after the infusion and normalized to the previous level around 45 minutes after the infusion. These were tested with the Wilcoxon signed rank test and were statistically significant ( $p < 0.01$ ).

The CEOAE profiles to an additive toxicity of the hypoxia on the loop diuretics could be deduced from the fact that additional exposure of the hypoxia immediately after the recovery from the ototoxic loop diuretic insult resulted in a more statistically significant decrease of the variables of the CEOAE than that of separate exposure to the ototoxic drug. Further comparative study with conventional acousticophysiological measures is mandatory to determine the underlying processes responsible for the synergistic effect.

The CEOAE, as one of the responses of the peripheral sense organ, has a perspective of developing into a functional cochleogram for evaluating cochlear lesions (Kemp *et al.* 1986; Bonfils and Uziel 1989; Bonfils *et al.* 1989). Changes in the echo level may not be a sign for differential diagnosis of cochlear pathology, but it remains as a subject of further study.

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