

Ototoxicity Resulting From Combined Administration of Metronidazole and Gentamicin

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Hypothesis: The hypothesis that metronidazole can augment the ototoxicity of gentamicin was tested.

Background: Metronidazole and gentamicin are antibiotics that are used in combination to provide broad-spectrum antimicrobial coverage. It has been observed clinically that an increased ototoxic effect occurs when these agents are used in combination.

Methods: Groups of guinea pigs were given various doses of gentamicin alone, various doses of gentamicin in combination with metronidazole, or metronidazole alone. Auditory damage was determined electrophysiologically by measurement of the compound action potential. Hair cell damage was quantified by immunofluorescent microscopy.

Results: Electrophysiologic data revealed an augmented ototoxic effect when metronidazole was given with both a moderate and a high dose of gentamicin. Thresholds (dB SPLp) for the compound action potential (N1) for animals receiving a medium dose of gentamicin alone (50 mg/kg) were approxi-

mately 20-dB SPLp. This threshold increased to approximately 50-dB SPLp when metronidazole (35 mg/kg) was administered along with the medium-dose gentamicin. Additionally, animals receiving high-dose gentamicin (75 mg/kg) alone demonstrated increased N1 thresholds from 85 to 95 when metronidazole (35 mg/kg) was added to the gentamicin regimen. This effect was evident histopathologically by increased cochlear hair cell damage. Outer hair cell loss for animals receiving medium-dose gentamicin alone did not differ from that of controls. When metronidazole (35 mg/kg) was combined, however, outer hair cell loss increased to approximately 50%.

Conclusions: These data support the clinical observation of augmented ototoxicity in patients receiving combined gentamicin and metronidazole. Caution should be used when administering these two agents together. Clinicians should consider other antibiotic strategies whenever possible. **Key Words:** Ototoxicity—Gentamicin—Metronidazole.

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Gentamicin is an aminoglycoside antibiotic commonly used in the treatment of infections caused by aerobic gram-negative bacteria. Adverse side effects have been well described and include nephrotoxicity and ototoxicity (1-3). The biochemical mechanisms for aminoglycoside ototoxicity consist of acute and chronic processes (4). Acute damage involves blockage of plasma membrane ion channels, whereas chronic damage is related to intracellular targets, such as phosphoinositides (5). Recent evidence suggests that iron chelation and free-radical formation are a part of the ototoxic actions of aminoglycosides (6).

Metronidazole is an antibiotic of the nitroimidazole class that is bactericidal for many anaerobic organisms. This agent reacts with iron-containing compounds within the organism to form toxic metabolites (7). There are no published reports of metronidazole-induced ototoxicity. Aminoglycosides such as gentamicin are often used in

conjunction with metronidazole to provide broad-spectrum antimicrobial coverage (8,9). It has been observed clinically that when these two agents are given together, an augmented ototoxic effect results (Hain TC. Personal communication, 1996).

Our purpose in the current study was to investigate the ototoxic effect of combined administration of metronidazole and gentamicin in the guinea pig. The dosage protocol included various amounts of gentamicin with and without metronidazole, or metronidazole alone. Although gentamicin ototoxicity can manifest as auditory or vestibular signs, this investigation focused on cochlear (auditory) damage by using standard physiologic and histologic techniques. Auditory damage was determined electrophysiologically by measurement of the compound action potential. Hair cell damage was quantified by cytochromeography using immunofluorescent microscopy. Attempts were made to correlate the degree of ototoxicity with the doses given and to draw conclusions regarding the potential augmented ototoxicity of these two drugs when they are given to the patient. The results of this investigation show that animals receiving a moderate dose of gentamicin in combination

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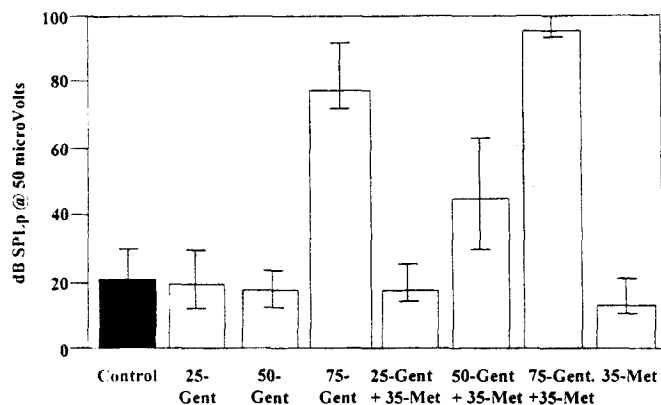


FIG. 1. Sound intensity required to elicit the compound action potential (N1) with 50 μV of peak amplitude. Brackets indicate 95% confidence intervals. Gent=Gentamicin (milligram/kilogram). Met=Metronidazole (milligram/kilogram). Thresholds were significantly higher for animals receiving combined medium-dose gentamicin with metronidazole than for animals receiving either medium-dose gentamicin alone or metronidazole alone.

with metronidazole had an increased amount of cochlear damage when compared with animals receiving either gentamicin or metronidazole alone.

MATERIALS AND METHODS

Dosage protocol

Female Duncan Hartley guinea pigs (range, 350–400 g) were used in this investigation. All animals were screened for auditory function by eliciting a Preyer’s pinna reflex. Forty animals were randomly assigned drug regimens (Table 1). The animals were weighed every other day and dosed accordingly. Groups I, II, and III received a once-daily, 14-day course of gentamicin alone, subcutaneously, in doses of 25 mg/kg, 50 mg/kg, and 75 mg/kg, respectively. Groups IV, V, and VI similarly received 14-day courses of gentamicin in doses of 25 mg/kg, 50 mg/kg, and 75 mg/kg, respectively. In addition, they received metronidazole (35 mg/kg) subcutaneously for 14 days. Group VII animals received a 14-day course of metronidazole alone (35 mg/kg), and group VIII animals received saline as the control group. After drug exposure, the animals were maintained over a 4-week period to allow cochlear damage to stabilize.

Methods for measuring ototoxicity

All guinea pigs were anesthetized with sodium pentobarbital (65 mg/kg) administered intraperitoneally. Body temperature was monitored with a rectal probe and maintained to 37° C to 38° C with a direct current heating pad. The bulla of each ear was exposed through a postauricular incision and opened to provide access to the round-window membrane.

Sound stimuli were presented to the ear through a calibrated Eytomotic ER-2 earphone (Eytomotic, Elk Grove Village, IL, U.S.A.). An insulated wire with a loop at the exposed end was gently placed on the round-window membrane using a micro-manipulator to the compound (N1) action potentials. Stimulus presentation and data acquisition were interfaced by a P5-133 Gateway 2000 computer and a Tucker-Davis Technologies (Gainesville, FL, U.S.A.) module. Round-window potentials were amplified with a Grass P511 preamplifier with the passband set to 0.3 kHz to 30 kHz and further amplified with a TST PC1 amplifier (Grass Medical Instruments, Quincy, MA, U.S.A.). Total amplification was at a gain of × 20,000. Round-window potentials were sampled through a 16-bit analog-to-digital converter (TDT AD1) at a sampling rate of 50 kHz. Average round-window potentials were generated in response to 100 presentations of the stimulus. Acoustic stimuli were digitally synthesized (TDT AP2 array processor card) and presented through a 16-bit digital-to-analog converter (TDT DA3-2) at a conversion rate of 50 kHz (TDT FT-5). Stimuli consisted of 60-μs clicks for evoking the compound action potential. All stimuli were presented once every 250 ms. Input-output functions for clicks were generated using 5-dB steps measuring the peak amplitude of the negative-going N1 potential at each sound level. Interval estimation techniques were used to statistically analyze the physiologic data, as shown in Figure 1, with brackets representing the 95% confidence interval for the sample means.

Each bulla was removed and dissected after the electrophysiologic study was completed. The cochleas were perfused through the round and oval windows with a solution of 4% paraaldehyde for 2 hours. They were rinsed and stored in 10% phosphate buffer

TABLE 1. Summary of treatment

Group	Animals/group	Gentamicin (mg/kg) × 14 days (mg/kg) × 14 days	Metronidazole (mg/kg) × 14 days
I	5	25	0
II	5	50	0
III	5	75	0
IV	5	25	35
V	5	50	35
VI	5	75	35
VII	5	0	35
VIII	5	Saline only	

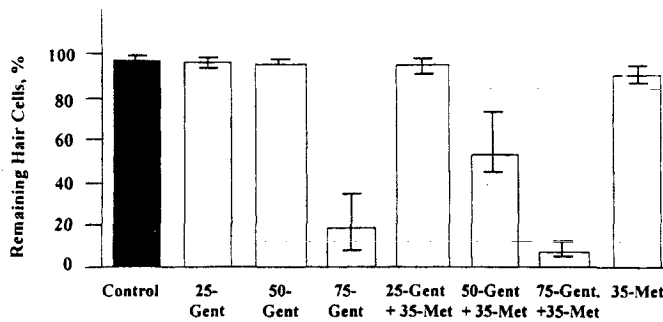


FIG. 2. Total percentage of remaining cochlear outer hair cells (PM1 standard deviation). Gent=Gentamicin (milligram/kilogram). Met=Metronidazole (milligram/kilogram). Hair cell loss was normal for animals receiving medium-dose gentamicin, but was significantly decreased in animals receiving a combination of medium-dose gentamicin and metronidazole.

solution and later stained with 1% rhodamine phalloidin (Molecular Probes, Eugene, OR, U.S.A.). The organ of Corti was microdissected from each cochlea and mounted on glass slides with Fluoromount (Fischer Scientific, Pittsburgh, PA, U.S.A.). Organ of Corti sections were viewed with a Nikon Fluorescent Microscope (Nikon, Melville, NY, U.S.A.). Outer hair cells were quantified from base to apex with the assistance of a computer monitor and recorded for statistical analysis. All histologic statistical data were analyzed by analysis of variance and are presented in Table 2.

RESULTS

The effect of the drugs on cochlear nerve activity was quantified for each animal by measurement of the compound action potential threshold (N1) for a 60- μ s click stimulus. The peak amplitude of the negative-going N1 was measured at each sound level (dB SPLp). The amount of sound (dB SPLp) required to elicit an N1 peak amplitude of 50 μ V was determined for each experimental group. The N1 data are shown in Figure 1. Thresholds for the compound action potential (N1) were significantly higher for animals receiving the drug combination of medium-dose gentamicin (50 mg/kg) with metronidazole (50-dB SPLp) than for animals receiving either metronidazole alone (15-dB SPLp) or a medium-dose gentamicin alone (20-dB SPLp). In addition, animals receiving high-dose gentamicin (75 mg/kg) in combination with metronidazole demonstrated higher thresholds (95-dB SPLp) than did animals receiving either metronidazole alone (15-dB SPLp) or high-dose gentamicin alone (85-dB SPLp). The remaining treatment groups did not differ significantly from the control group (20-dB SPLp). Figure 2 summarizes the input-output functions for all eight groups. The graphs show how the compound action potential (N1) amplitude changes as the sound intensity (dB SPLp) increases.

Cochlear outer hair cell damage was quantified by immunofluorescent microscopic examination from base to apex. Figure 3 summarizes the percentage of outer hair cells remaining for each group. Damage was most severe for animals receiving high-dose gentamicin (75 mg/kg) with metronidazole, with total hair cell loss of 95%. Damage was significant at both the base (100%) and the apex (85%) of the cochlea. Animals receiving high-dose gentamicin alone had hair cell losses of 80% overall. Damage

was found to be most severe at the basal turn (100%) and least severe at the apex (85%) for this group. Animals receiving a medium dose of gentamicin (50 mg/kg) together with metronidazole also experienced significant hair cell loss of approximately 50%. Losses ranged from 60% at the apex to 50% at the base of the cochlea. The remaining animal groups had outer hair cell populations that did not differ significantly from those of control groups.

DISCUSSION

Concomitant use of metronidazole and gentamicin as broad-spectrum antimicrobial treatment is a common clinical strategy (8,9). In this study, we investigated the potential for augmented ototoxicity when these two agents are used together. Preliminary clinical evidence supports this possibility with vestibular testing on patients who had received concomitant metronidazole and gentamicin therapy. Our experimental model, however, examined cochlear damage by using reliable techniques that compare drug ototoxicity. Correlations with vestibular damage, although likely, were not examined in this study.

Both our electrophysiologic and our histopathologic data support the clinical observation of augmented ototoxicity with combined metronidazole and gentamicin. Auditory damage was assessed with the compound action potential (N1), which is a measure of cochlear nerve activity. Thresholds for the N1 potential were significantly higher for animals receiving combined gentamicin and metronidazole when medium and high doses of gentamicin were used.

Structural changes in the cochlea were quantified by outer hair cell counts. In agreement with the electrophysiologic data, damage was greatest in animals receiving metronidazole and high-dose gentamicin. The most significant difference, however, was seen in comparison of the combined medium-dose gentamicin and metronidazole with either medium-dose gentamicin alone or metronidazole alone. The combined group demonstrated hair cell losses of 50%, whereas the remaining groups had losses similar to those of control groups (5%). Not only was the amount of hair cell loss greater for the combined groups, but the distribution of the loss was different as well. Animals receiving combined regimens had proportionally higher damage in the apex of the cochlea.

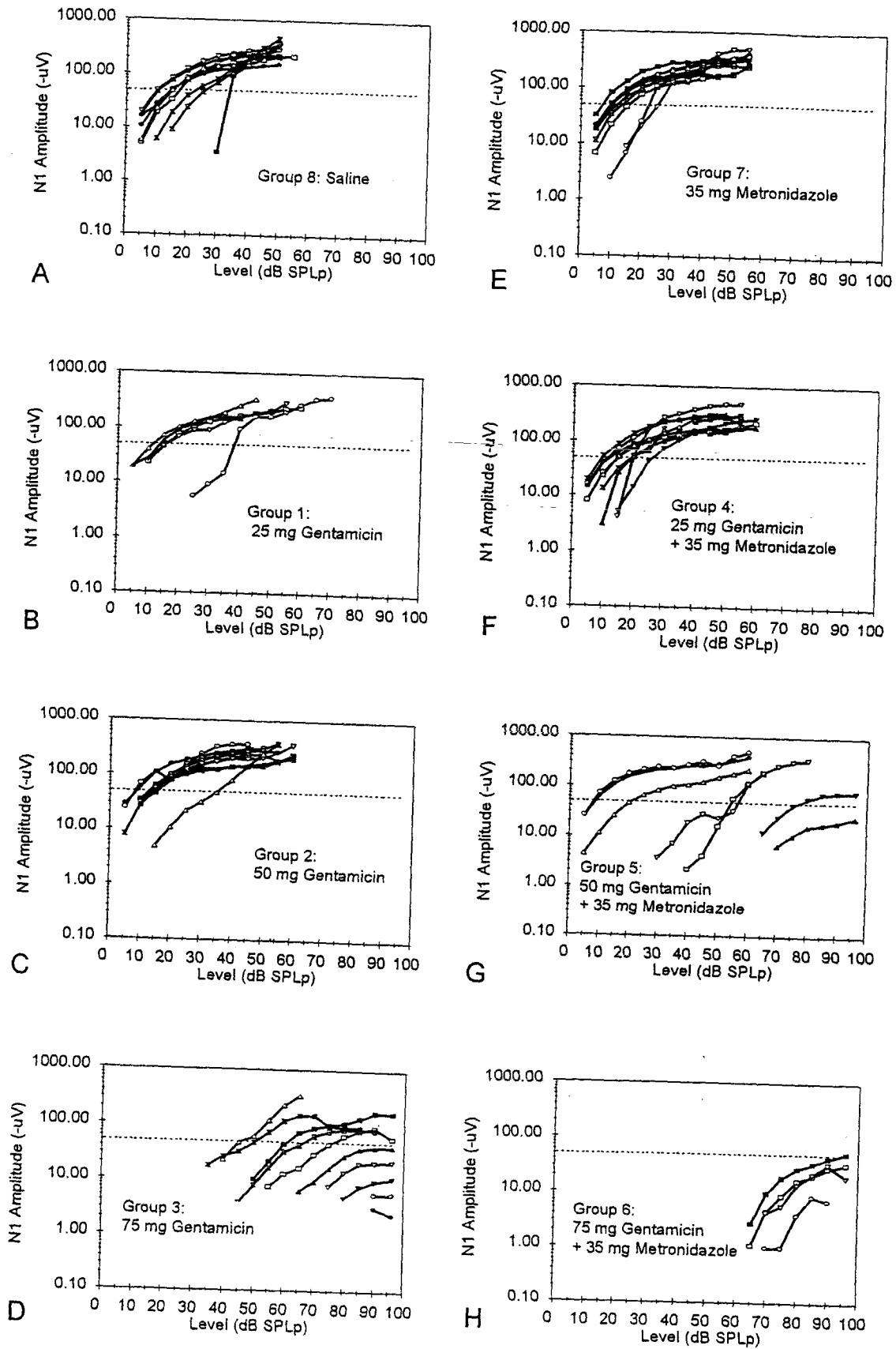


FIG. 3. Input-output functions for all animal groups. Each function is from a different ear. Horizontal dotted line in each graph shows the N1 amplitude at $-50 \mu\text{V}$, defined as threshold. X-axis denotes the amount of sound (dB SPLp) needed to achieve threshold.

In contrast, animals receiving gentamicin alone had the greatest damage in the basal turn, which is typical for aminoglycoside ototoxicity.

CONCLUSION

We believe that metronidazole can augment the ototoxicity of gentamicin. The mechanism by which this effect occurs is unknown. Metronidazole alone has not been shown to be ototoxic. It appears likely, however, that metronidazole acts to potentiate the ototoxicity of gentamicin. The biochemical mechanism of aminoglycoside ototoxicity is thought to involve blockage of plasma membrane ion channels and targeting of cell signal mediators, such as phosphoinositides (5). Recent evidence suggests that iron chelation and free-radical production are part of the ototoxic mechanism of aminoglycosides. The presence of gentamicin is thought to enhance iron-catalyzed oxidation and free-radical formation (6). Interestingly, the antimicrobial mechanism of metronidazole involves iron compounds as well, in which it is chemically reduced by ferredoxin to form byproducts toxic to the organism (7). Perhaps this mutual reactivity with iron is responsible for the augmented ototoxic effect of combined gentamicin and metronidazole. Further investigation is needed to identify this mechanism.

Based on the data presented here, the combination of metronidazole and gentamicin should be used with caution. The therapeutic index of aminoglycosides is already low. The combination of gentamicin with metronidazole appears to have an even lower index of safety. The clinician should consider alternative antibiotic strategies whenever possible.

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