

The Effects of Four, Commercial Ceruminolytic Agents on the Middle Ear

Four, commercially available ceruminolytic agents and physiological saline were screened for ototoxic and inflammatory reactions on the middle ear mucosae of guinea pigs (n=38) and dogs (n=24). Each solution was injected transtympanically in anesthetized animals. The effects were assessed by brain stem auditory evoked response (BAER) tests to evaluate hearing function and by histological examination of the middle ear structures. Varying degrees of hearing loss and inflammation were observed in some guinea pigs and dogs treated with solutions A, C, and D, whereas no abnormal finding was associated with solution B or saline.

J Am Anim Hosp Assoc 1997;33:479-86.

Philip D. Mansfield, DVM

Janet E. Steiss, DVM, PhD

Timothy R. Boosinger, DVM, PhD

Arvle E. Marshall, DVM, PhD



Introduction

Topical otic preparations containing a variety of substances are used routinely in the treatment of animals with otitis externa. It has been reported that the tympanum is ruptured in 50% of dogs affected with chronic otitis externa.¹ When rupture of the tympanic membrane occurs (secondary to infection, inflammation, or both; when a myringotomy is performed; or if accidental perforation occurs), topically applied substances can enter into the middle ear cavity and be absorbed by the mucosa and submucosa.² Many of these substances are potentially ototoxic.³⁻⁷ These preparations may cause inflammation that can result in conductive hearing loss, or they may pass through the cochlear (i.e., round) window into the inner ear and cause sensorineural hearing loss, injury to sensory cells of the vestibular apparatus, or both.^{2,3} Regardless of the site of injury, risk may be associated with the use of topical agents in the external ear canal when the tympanic membrane is not intact.

Examination of the external meatus and tympanic membrane of the inflamed ear cannot be completed, and effective treatment cannot be accomplished if the canal is occluded with cerumen or exudate. Cleaning often is performed using agents that contain surface-active substances which soften and emulsify the wax and lipids in the ear canal. The application of these ceruminolytic agents to the external ear canal has been reported to be contraindicated if the tympanic membrane has been perforated.^{8,9} Although no safety studies of ceruminolytic agents have been published, some manufacturers warn that their products should not be used if the tympanic membrane is perforated. A warning accompanies two of the four commercial solutions selected for this study.

Ingredients of ceruminolytic agents differ among manufacturers but generally consist of squalene, dioctyl sodium sulfosuccinate, dioctyl calcium sulfosuccinate, triethanolamine polypeptide oleate-condensate, or carbamide peroxide. One or more of these substances will be in an alcohol, chlorobutanol, isopropyl myristate, propylene glycol, glycerine, or water base.

In this study, the safety of four, commercial ceruminolytic agents was studied in guinea pigs and dogs [Table 1]. Each solution contained different surface-active agents [Table 2]. The focus of the

From the Departments of Small Animal Surgery and Medicine (Mansfield), Scott-Ritchey Research Center (Steiss), Pathobiology (Boosinger), and Anatomy and Histology (Marshall), College of Veterinary Medicine, Auburn University, Auburn, Alabama 36849-5523.

Table 1
Description of Ceruminolytic Solutions Tested on Guinea Pigs and Dogs

Solution	Ingredients
A ^{a*}	Glycerine USP, aloe vera gel, tea lauryl sulfate, dioctyl sodium sulfosuccinate (2%), parachlorometaxyleneol, fragrance, and FD&C blue #1
B ^b	Squalene (25%) and isopropyl myristate with liquid petrolatum base
C ^c	Carbamide peroxide (6.0%) and dioctyl sodium sulfosuccinate (6.5%) in propylene glycol
D ^d	Triethanolamine polypeptide oleate-condensate (10%), chlorobutanol (0.5%), propylene glycol, and water

* The formula for solution A has changed since this study was performed. Currently the ingredients are purified water USP, isopropyl alcohol, aloe vera, diazolidinyl urea, methylparaben, propylparaben, dioctyl sodium sulfosuccinate, octoxynol, sodium lauryl sulfate, parachlorometaxyleneol, propylene glycol USP, fragrance, tetrasodium EDTA, and FD&C blue #1

Table 2
Surface-Active Agents in Ceruminolytic Solutions¹⁰

Agent	Properties
Squalene:	[2,6,10,15,19,28-Hexamethyl-2,6,10,14,18,22-tetracosahexaene]. Used as a surface-active agent. It is bactericidal, has a faint agreeable odor, absorbs oxygen, and becomes slightly viscous on application. Squalene is practically insoluble in water. It is readily soluble in ether, acetone, and carbon tetrachloride.
Dioctyl sodium sulfosuccinate:	[Sulfobutanedioic acid 1,4-bis (2 ethylhexyl) ester sodium salt]. Used as a wetting agent. This agent is soluble in water, carbon tetrachloride, ether, naphtha, xylene, acetone, alcohol, and vegetable oil.
Carbamide peroxide:	[CH ₄ N ₂ O ₃]. When exposed to air and moisture, carbamide peroxide is reduced to urea, oxygen, and water. The effervescence caused by the release of the oxygen facilitates the removal of debris and produces a weak antibacterial effect.
Triethanolamine:	[2,2',2"-Nitrilotrisethanol]. A very hygroscopic, viscous liquid that is used in making emulsions with mineral and vegetable oils and waxes. Miscible with water, methanol, and acetone.

study was on the effects of these solutions on changes in the brain stem auditory evoked response (BAER) and on the mucosal lining of the middle ear.

The BAER is an objective electrodiagnostic test which can be used to evaluate the functional integrity of the auditory nerve (hence, cochlear structures) and the brain stem auditory pathways. The threshold for the BAER correlates with behavioral hearing thresholds.¹¹ Therefore, an elevated BAER threshold provides a reasonable indication of an elevated hearing threshold. Partial hearing loss results in increased thresholds and response latencies and in characteristic shifts in latency-intensity curves of the BAER. Severe or complete hearing loss results in the absence of a response.

Materials and Methods

Thirty-eight young, healthy, white, male guinea pigs (weighing 300 to 350 g) were obtained from a laboratory animal source. Before being assigned randomly to treatment groups, the auditory function of each animal was assessed by BAER performed under anesthesia (as described below). Only animals judged to exhibit a normal BAER were selected.

The guinea pigs were divided randomly into five groups. One ear of each guinea pig was treated with solution A^a (n=12), solution B^b (n=6), solution C^c (n=6), solution D^d (n=6), or sterile 0.9% saline (n=8). The saline-treated ears served as controls. The contralateral ear of each guinea pig (n=38) was not treated. All solutions were cultured at the beginning

and end of the study to assure that they were free of contaminating bacteria.

The guinea pigs were anesthetized by intramuscular (IM) injection of fentanyl (0.4 mg/ml) and droperidol^e (20 mg/ml) at a dose of 0.5 ml/kg body weight. This was followed in five minutes by atropine sulfate (0.04 mg/kg body weight, IM) and an intraperitoneal injection of pentobarbital sodium (25 mg/kg body weight). The tympanic membrane of one randomly selected ear of each animal was visualized using a 3-mm otoscopic speculum. A 22-gauge, 6.35-cm sterile needle^f was passed through the speculum, the pars tensa was perforated, and 0.2 ml of one of the test solutions was instilled into the tympanic cavity.

On days zero, two, seven, 14, and 28 after treatment, a modified neurological examination was performed which included assessment of gait, head tilt, spontaneous and positional nystagmus, and righting and postural responses.

On days zero, 14, and 28 after treatment, the guinea pigs again were anesthetized using the previously described protocol, and BAER tests were performed. The recording procedure was based on methods previously described for the dog.¹² The click stimulus was applied through ear insert tubes. Signal-averaged responses^g were recorded from each ear starting with the highest intensity level of 90 decibels sound pressure level (dB SPL), which was decreased stepwise by 20 decibels (dB) to the observed response threshold (defined as the first appearance of wave V). The threshold then was determined to be within 10 dB SPL. Latencies for waves I through V were measured with an automatic cursor display on the monitor.

On day 29, the animals were anesthetized with pentobarbital sodium and exsanguinated. The petrosal and squamous parts of the temporal bone were cut with a bone saw; the middle and inner ears were removed; and the tympanic bullae of both ears were opened. If inflammation in the middle ear was not severe enough to prevent identification of the stapes, this structure was removed to open the vestibular (i.e., oval) window. A second opening was made at the apex of the cochlea. Through these openings, each inner ear was perfused with Bouin's solution. The tissues were fixed, decalcified with ethylenediaminetetraacetic acid (EDTA), embedded in paraffin wax, sectioned at 7 μ m, stained with hematoxylin and eosin stain, and examined by light microscopy. The treated ear was compared to the contralateral untreated ear from the same animal.

For the second phase of the study, 24 (14 males and 10 females) healthy, adult, random-source, mixed-breed dogs were selected on the basis of normal otoscopic examinations and normal BAER tests performed under anesthesia using fentanyl-droperidol (0.1 ml/kg body weight, IM), pentobarbital sodium

(25 mg/kg body weight, intravenously [IV]), and atropine (0.04 mg/kg body weight, IM). They were divided randomly into four treatment groups with each group (n=6) receiving one of the same solutions used in the guinea pig trials [Table 1]. The dogs were treated and evaluated in the manner described for the guinea pigs, with the following exceptions:

- 1) Within each treatment group, half of the animals were treated in the contralateral ear with sterile 0.9% saline and the remaining half were untreated controls.
- 2) One ml of test solution was instilled in the perforated middle ear.

Results

Saline-Treated Controls

Brain stem auditory evoked response thresholds ranged from 10 to 30 dB SPL for the eight guinea pig ears and the 12 canine ears except for one dog that exhibited a threshold response of 50 dB SPL on day 28. All saline-treated ears were grossly and microscopically normal on day 29. No inflammatory reaction was associated with the method of treatment.

Untreated Controls

Throughout the portion of the study using guinea pigs, 31 of the 38 contralateral ears that served as untreated controls exhibited normal BAERs. Seven had increased BAER thresholds of 50 dB SPL on day 28. Of these seven ears, two were from guinea pigs treated with solution A, four were from guinea pigs treated with solution B, and one was from a guinea pig treated with saline. No gross or microscopic abnormalities were observed in any of the 38 untreated ears.

All untreated canine ears remained normal throughout the study, except for one dog which exhibited a threshold that was elevated to 50 dB SPL on day 28.

Solution A-Treated Guinea Pigs

On day two after treatment, eight of 12 guinea pigs each exhibited a head tilt toward the treated side. Five of these animals had head tilts on day seven, but only one had the head tilt on days 14 and 28. Five of the animals that had head tilts on day two also exhibited nystagmus. Spontaneous horizontal movement was present in one; four others demonstrated horizontal nystagmus when positioned on their backs. In each case, the fast phase was directed away from the treated side. Nystagmus was not present on subsequent examinations. No other neurological abnormality was observed.

Nine of 12 guinea pigs treated with solution A had absent BAERs from the treated ears on day 14. Eleven

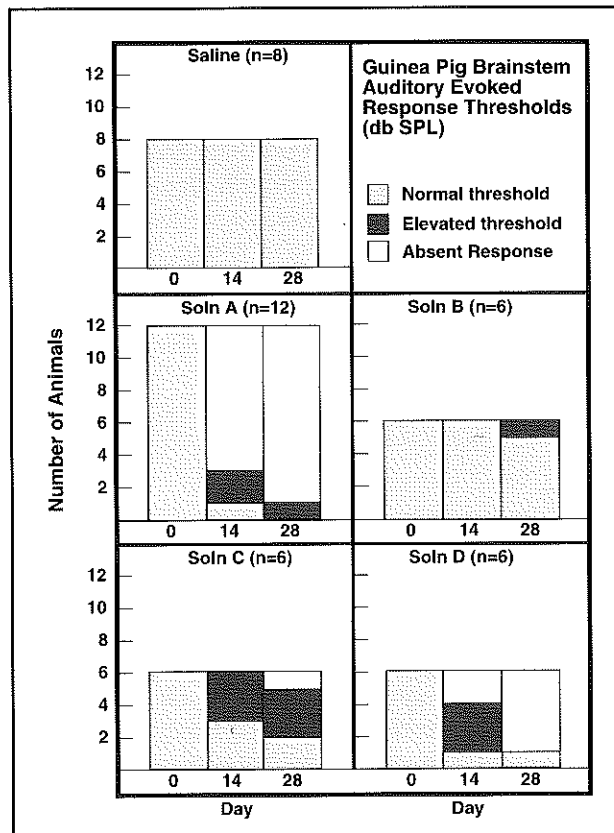


Figure 1—Histograms for thresholds (decibels sound pressure level [dB SPL]) for the brain stem auditory evoked responses recorded from the ears of guinea pigs which received transtympanic treatment with test solution (soln) A, B, C, or D or with sterile physiological saline. Day zero is pretreatment; days 14 and 28 are posttreatment. Normal thresholds, as measured on day zero, ranged up to 30 dB SPL. "Elevated threshold" is defined as increased above the pretreatment value but present within the stimulus intensity range tested. "Absent response" is defined as no response to any intensity up to and including the maximum of 90 dB SPL.

had no response from the treated ears on day 28, and the remaining animal had an elevated BAER threshold from the treated ear on day 28 [Figure 1, Solution A].

On necropsy, all guinea pigs treated with solution A had gross morphological changes in the treated ears. In 10 animals, the osseous bullae were thickened markedly on the treated sides, and the tympanic cavities were filled completely with thick, mucoid-like secretions [Figure 2]. No secretions were present in the tympanic cavities of two animals, but the auditory ossicles in these ears appeared to be fused and covered with granulation tissue.

Inflammation in the bullae was characterized by necrotic, cellular debris and neutrophils that filled the tympanic cavities. Fibrous connective tissue lined the cavities of the middle ear. Fibroblasts and leukocytes extended into adjacent bone, consistent with chronic osteomyelitis. Small bacterial colonies were present in some of the inflamed bullae. No remarkable microscopic changes were present in one solution A-treated ear.

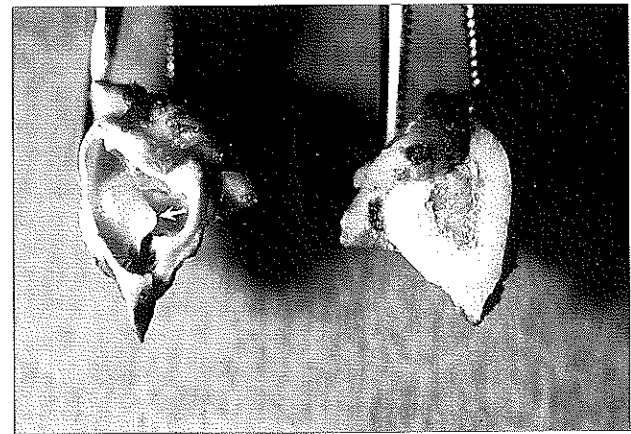


Figure 2—Solution A-treated guinea pig ear (right) shown with contralateral untreated control. Identical cuts have been made in the osseous bullae in order to provide the same exposure to the tympanic cavity of each ear. The cochlea of the control ear can be seen (arrow) in its normal position as it protrudes into the cavity of the middle ear.

The cochlea of the guinea pig is not embedded deeply in the temporal bone as it is in other species. Instead, it protrudes into the cavity of the middle ear. Consequently, the outer surfaces of the cochleae in the inflamed ears were covered by exudate. Only one solution A-treated ear exhibited inflammation within the cochlea. The neurosensory linings of all cochleae were intact and morphologically normal.

Solution A-Treated Dogs

On day two after treatment, one of six dogs had a slight ipsilateral head tilt. This sign was absent on day seven. No other neurological abnormality was observed.

In two of the six solution A-treated ears, the BAER thresholds were elevated above 30 dB SPL on days 14 and 28, indicating partial hearing loss. One dog had no response at the highest intensity tested in the treated ear on days 14 and 28 [Figure 3, Solution A].

All six ears exhibited gross morphological changes within the middle ear at necropsy. The osseous bullae had marked thickenings, and the tympanic cavities were filled with fibrous tissue and mucoid-like secretions.

Microscopically, severe inflammatory reaction and associated morphological changes were observed in these ears [Figures 4A, 4B]. Lymphocyte and macrophage infiltration resulted in near complete effacement of all middle and inner ear structures. The changes were complicated further by severe inflammation of approximately 2 mm of bone surrounding the middle ear. The osteomyelitis was characterized by a zone of fibrous tissue proliferation plus the presence of macrophages, lymphocytes, and osteoclasts.

Solution B-Treated Guinea Pigs

None of the six guinea pigs treated with solution B had any abnormality on neurological or histological

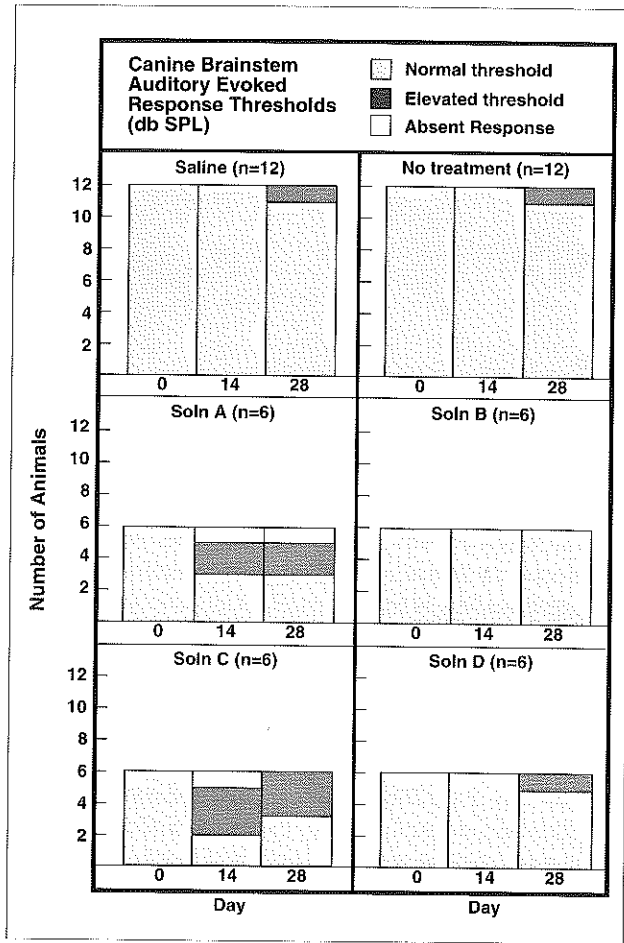


Figure 3—Histograms for thresholds (decibels sound pressure level [dB SPL]) for the brain stem auditory evoked responses recorded from the ears of dogs which received transtympanic treatment with test solution (soln) A, B, C, or D or from the control ears which received either sterile physiological saline or no treatment. Day zero is pretreatment; days 14 and 28 are posttreatment. Normal thresholds, as measured on day zero, ranged up to 30 dB SPL. "Elevated threshold" is defined as increased above the pretreatment value but present within the stimulus intensity range tested. "Absent response" is defined as no response to any intensity up to and including the maximum of 90 dB SPL.

examination. One of these animals had an increased BAER threshold in the treated ear on day 28 [Figure 1, Solution B], but this animal and three others in this treatment group had similar BAER threshold changes in their contralateral untreated ears. The reason for this is unknown.

Solution B-Treated Dogs

None of the dogs treated with solution B exhibited functional or morphological changes [Figure 3, Solution B].

Solution C-Treated Guinea Pigs

None of the six solution C-treated guinea pigs had a neurological abnormality. On day 14, three animals each had increased BAER thresholds in the treated ears. On day 28, one of these had advanced to a total absence of

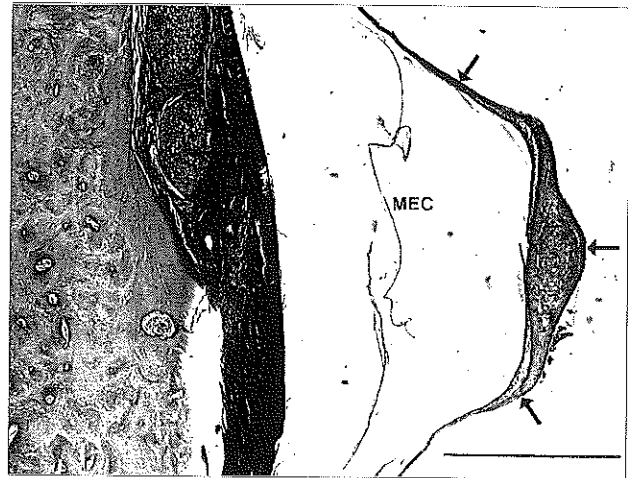


Figure 4A

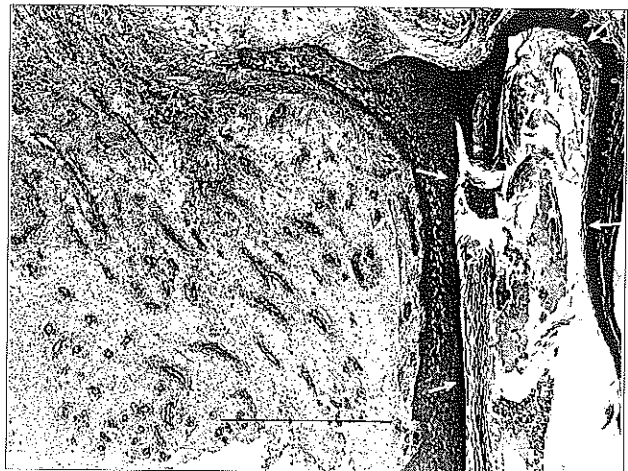


Figure 4B

Figures 4A, 4B—(A) A photomicrograph of a normal canine middle ear cavity (MEC) including the tympanic membrane (delineated by black arrows) from an untreated control (Hema-toxylin and eosin stain, 50X; bar=0.5 mm). (B) A photomicrograph of a site-matched section from a canine ear treated transtympanically with solution A. Squamous epithelium of the middle ear is separated from underlying bone by a diffuse zone of leukocytes and neovascularization. The middle ear cavity (delineated by white arrows) is filled with keratin and leukocytes. The tympanic membrane could not be identified (Hema-toxylin and eosin stain, 50X; bar=0.5 mm).

BAER; two of the three guinea pigs continued to exhibit increased thresholds; and a fourth had an increased BAER threshold [Figure 1, Solution C].

Three of the treated ears had thickened bullae; in one of these, an infiltration of leukocytes filled the middle ear cavity. No microscopic lesions were identified in five solution C-treated specimens.

Solution C-Treated Dogs

On day two after treatment, one solution C-treated dog exhibited a severe ipsilateral head tilt, with spontaneous horizontal nystagmus which markedly in-

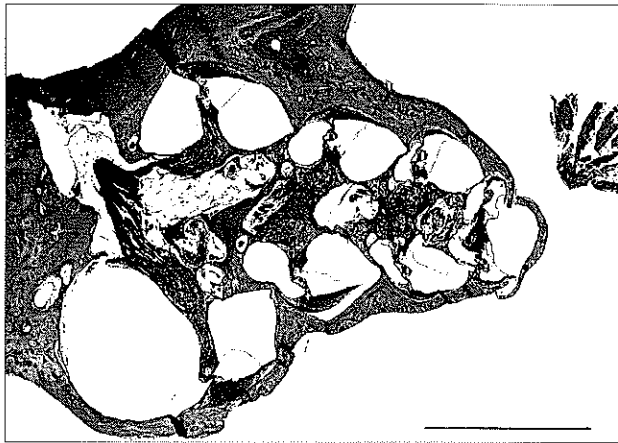


Figure 5A

Figures 5A, 5B—(A) A photomicrograph of a normal guinea pig cochlea from an untreated control (Hematoxylin and eosin stain, 25X; bar=1.0 mm). (B) A photomicrograph of a site-matched section from a guinea pig ear treated transtympanically with solution D. The inflamed cochlea partially is filled with and surrounded by diffuse sheets of leukocytes and cellular debris (Hematoxylin and eosin stain, 25X; bar=1.0 mm).

creased when the animal was positioned in dorsal recumbency. The dog could not maintain balance when the postural reactions were tested. These neurological abnormalities remained constant throughout the study. The BAER thresholds for this dog's treated ear were 50 and 70 dB SPL on days 14 and 28, respectively. Histologically, mild otitis media was identified, but there was no osteomyelitis nor remarkable changes in the inner ear.

On day 14, the BAER thresholds were increased above 30 dB SPL in three of the solution C-treated ears and BAER was absent in one of the ears. On day 28, the BAER was normal in one of the ears that previously had shown an increased threshold. In the ear that previously had shown no response, the BAER was present at an increased threshold. On day 28, three dogs had elevated BAER thresholds and three had normal BAERs [Figure 3, Solution C].

On necropsy, gross morphological changes were evident in all six treated ears. In one ear, the auditory ossicles were sclerotic; they were so altered as to make their identification impossible. The osseous bulla of another ear was thickened markedly, and the cavity of the middle ear was filled with a caseous material. The changes observed in four other ears consisted of what appeared to be fibrous tissue lining the tympanic cavities and covering the ossicles. The microscopic lesions affecting all the solution C-treated canine ears consisted of mild inflammatory changes characterized by proliferation of fibrous connective tissue and lymphocytic infiltration.

Solution D-Treated Guinea Pigs

On days two, seven, and 14, one guinea pig treated with solution D had an ipsilateral head tilt. On day



Figure 5B

two, nystagmus was observed when this animal was positioned in dorsal recumbency. No other neurological abnormality was observed, and the animal appeared behaviorally normal on day 28.

On days 14 and 28, two of the animals in this treatment group had absent BAERs in the treated ears. Three others had increased BAER thresholds on day 14, and there were no BAERs from the treated ears on day 28. One animal in this treatment group maintained a normal BAER [Figure 1, Solution D].

On necropsy, three of the ears had marked thickenings of the osseous bullae, and the tympanic cavities were filled with thick, mucoid-like material. The remaining three ears appeared to have minor bony changes affecting the bullae and the auditory ossicles.

Severe otitis media and interna were evident microscopically [Figures 5A, 5B]; osteomyelitis affected two ears. Moderate otitis media was observed in one ear; mild inflammatory changes were seen in two ears; and no morphological change was seen in one ear.

Solution D-Treated Dogs

No neurological abnormalities were observed in this group of dogs. One of the ears had an increased BAER threshold on day 28 [Figure 3, Solution D].

Two of the canine ears treated with solution D were grossly normal at necropsy. The osseous bullae of four ears were thickened, and the cavities contained a thick, mucoid-like material [Figure 6].

Microscopically, severe otitis and osteomyelitis were evident in one treated ear. Four others were characterized by mild-to-moderately severe, diffuse otitis, and one specimen showed no remarkable change.

Discussion

This study demonstrated that some commercially produced ceruminolytic agents cause inflammation and partial-to-complete loss of cochlear function when

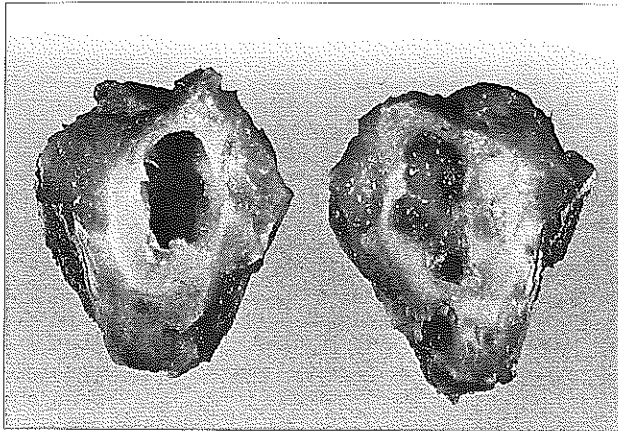


Figure 6—Solution D-treated canine ear (right) shown with the contralateral untreated control. Identical cuts have been made in the osseous bullae in order to provide the same exposure to the tympanic cavity of each ear.

used under conditions that permit their entry into the middle ear cavity. This information has practical relevance because ceruminolytic agents commonly are used in cleaning the ear canal that is occluded with cerumen or exudate. The occlusion prevents adequate examination of the tympanic membrane. Even when the tympanic membrane can be visualized with an otoscope, assessment is difficult. One cannot be certain that the tympanic membrane is intact unless pneumatic otoscopy or tympanometry is performed.

Topical preparations that are used in the external ear canal should be tested for inflammatory properties and for ototoxicity which might lead to changes in morphology or function of the middle ear, inner ear, or both. No such tests have been reported on the effects of the solutions used in this study. However, other agents and some components of the solutions tested in this study have been reported to have inflammatory effects on middle ear mucosa.^{2,7} For example, propylene glycol, a component of solutions C and D (and currently an ingredient of the reformulated solution A), has been reported to induce inflammation of the middle ear mucosa and to cause neurosensory cell loss.^{3,6,7} On the basis of the current study, one cannot say whether the propylene glycol or other components of these solutions were responsible for the inflammatory changes observed.

Inflammation of the middle ear generally is known to cause a conductive hearing loss; however, some clinical studies have reported sensorineural hearing loss following otitis media.^{2,7} If severe middle ear inflammation persists, combined conductive and sensorineural deafness may result.² In the inner ear, fibrotic changes may occur,¹³ but the primary effect of ototoxic drugs is injury or destruction of the sensory receptors (i.e., hair cells) of the cochlea and vestibular apparatus.³⁻⁵ Injury to neurosensory cells was not documented in this study.

It was reported in a previous study that a single puncture of the tympanic membrane, such as that used to instill the test solutions into the tympanic cavity, resulted in no elevation in BAER threshold in dogs.¹⁴ The BAER threshold changes that occurred in this study could be explained by inflammation in the middle ear causing conductive losses, but total absence of a BAER at 90 dB SPL as seen in several of the test animals was suggestive of a loss of cochlear function. Inner ear changes are the subject of further study.

An explanation cannot be provided for the increased BAER thresholds that occurred in seven of the guinea pig ears that served as untreated controls. It is improbable that the elevated threshold resulted from the absorption of treatment agents applied to the contralateral ear. The anesthetic agents were the only other substances administered to any animal, and these drugs were chosen because they have been reported to have no ototoxic effects in guinea pigs.¹⁵ No morphological evidence of spontaneously occurring otitis was observed in these ears. Also, one guinea pig treated with solution A, one treated with solution B, and three treated with solution C had elevated BAER thresholds with no evidence of morphological changes in the middle ear cavity.

Conclusion

Inflammatory changes were observed in the ears treated transtympanically with solutions A, C, and D. These inflammatory reactions in the middle ear were associated with adverse effects on the BAER. Both guinea pigs and dogs were affected similarly, suggesting that animals of different species are at risk of adverse effects on hearing if these substances gain entry into the middle ear. These ceruminolytic agents should not be applied to the external ear canal when the tympanic membrane may be perforated.

^a Panotic; Pfizer Inc., Exton, PA

^b Cerumene; Evsco Pharmaceuticals, Immunogenetics, Inc., Buena, NJ

^c ClearX; Dermatologics for Veterinary Medicine, Inc., Miami, FL

^d Cerumenex; The Purdue Frederick Co., Norwalk, CT

^e Innovar-Vet; Pitman-Moore Inc., Mundelein, IL

^f Spinal needle 405074; Becton Dickinson Co., Franklin Lakes, NJ

^g Model TD 20; Teca Corp., Pleasantville, NY

Acknowledgments

Supported in part by the American Animal Hospital Association Foundation and the Scott-Ritchey Research Center.

References

1. August JR. Otitis externa, a disease of multifactorial etiology. *Vet Clin N Am Sm Anim Pract* 1988;18:731-42.

(Continued on next page)

References (cont'd)

2. Morizono T. Toxicity of ototoxic drugs: animal modeling. *Ann Oto Rhinol Laryngol* 1990;99:42-5.
 3. Rohn GN, Meyerhoff WL, Wright CG. Ototoxicity of topical agents. *Otolaryngol Clin N Am* 1993;26:747-58.
 4. Pickrell JA, Oehme FW, Cash WC. Ototoxicity in dogs and cats. *Semin Vet Med Surg (Sm Anim)* 1993;8:42-9.
 5. Mansfield PD. Ototoxicity in dogs and cats. *Comp Cont Ed Pract Vet* 1990;12:331-77.
 6. Merchant SR. Ototoxicity. *Vet Clin N Am Sm Anim Pract* 1993;24:971-80.
 7. Parker FL, James GWL. The effects of various topical antibiotic and antibacterial agents on the middle and inner ear of the guinea pig. *J Pharm Pharmacol* 1978;30:236-9.
 8. Griffin C. Principles for treatment of the diseased ear canal. In: *The complete manual of ear care*. Lawrenceville, NJ: Veterinary Learning Systems, 1986:61-5.
 9. Woody BJ, Fox SM. Laying the groundwork of disease management. *Vet Med* 1986;81:607-14.
 10. Budavari S. *The Merck index, an encyclopedia of chemicals, drugs, and biologicals*. 12th ed. Rahway, NJ: Merck and Co, 1996:575-1683.
 11. Van der Drift JFC, Brocaar MP, van Zanten GA. The relationship between the pure-tone audiogram and the click auditory brainstem response threshold in cochlear hearing loss. *Audiology* 1987;26:1-10.
 12. Steiss JE, Boosinger TR, Wright JC, Storrs DP, Pillai SR. Healing of experimentally perforated tympanic membranes, demonstrated by electrodiagnostic testing and histopathology. *J Am Anim Hosp Assoc* 1992;28:307-10.
 13. Galle HG, Vander-van Haagen AJ. Ototoxicity of the antiseptic combination chlorhexidine/centrimide (savlon): effects on equilibrium and hearing. *Vet Q* 1986;8:56-60.
 14. Steiss JE, Wright JC, Storrs DP. Alterations in the brain stem auditory evoked response threshold and latency-intensity curve associated with conductive hearing loss in dogs. *Prog Vet Neurol* 1990;1:205-11.
 15. McCormick JG, Nuttal AL. Auditory research. In: Wagner JE, Manning PT, eds. *The biology of the guinea pig*. New York: Academic Press, 1976:281-303.
-