Otology & Neurotology
35:743–747 © 2014, Otology & Neurotology, Inc.

Ototoxicity of Gentian Violet on the Guinea Pig Cochlea

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Purpose: Gentian violet (GV) is an antimicrobial and antifungal agent that has been used widely to treat intractable discharge in the ear. The purpose of this report is to warn clinicians about the ototoxic effect of GV in the middle ear.
Materials and Methods: GV ototoxicity was evaluated by measuring compound action potentials (CAPs) in the VIIIth nerve in adult Hartley guinea pigs. The middle ear cavities of the animals were filled with GV solution (0.5% or 0.13%), and CAPs were measured after intervals of 5 and 30 minutes and 1, 2, 6, and 24 hours. After all measurements were completed, the temporal bones were harvested for histopathologic evaluation. Celloidin-embedded specimens were cut into 20-μm slices and examined using light microscopy. The bacteriostatic activity of GV was evaluated using a disk-diffusion assay.
Results: A 0.5% GV solution produced a mild elevation in the CAP threshold at 30 minutes, a greater reduction at 1 hour, and complete abolishment of CAP at 24 hours. A 0.13% GV solution caused mild elevation in the CAP threshold at 2 hours and severe elevation at 6 hours. Massive new bone formation was found in the middle ear cavity at 6 weeks. GV concentrations of 0.13% and 0.06% were effective against all bacteria tested, with the exception of *Pseudomonas aeruginosa*.
Conclusions: Although GV has marked antibacterial and antifungal activities, its use should be limited to the external ear canal. GV exerts an ototoxic effect in a concentration- and time-dependent manner, and so the use of this drug in the middle ear cavity is not recommended. **Key Words:** Gentian violet—Guinea pig—Ototoxicity.
Otol Neurotol **35:**743–747, 2014.

The antibacterial and antifungal activity of gentian violet (GV) has been known for more than 60 years. Empirical GV treatment of chronic otitis externa by otologists worldwide indicates its therapeutic usefulness. However, to our knowledge, GV ototoxicity in the middle ear has not been determined. We examined the ototoxic effects of GV in the guinea pig cochlea by measuring compound action potentials (CAPs) in the VIIIth nerve. In addition, we evaluated the bacteriostatic activity of GV and the histopathologic changes in the temporal bone of GV-treated guinea pigs.

MATERIALS AND METHODS

Animals

Albino Hartley guinea pigs were used to evaluate the ototoxicity of GV. All animals had positive Preyer's reflex. The study protocol was approved by the Fukuoka University animal ethics committee.

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The authors disclose no conflicts of interest.

Anesthetics

The animals were anesthetized with sodium pentobarbital (30 mg/kg) and were secured in a custom-made head holder. Xylocaine (0.5%) was infiltrated into the surgical area before making a skin incision for access to the middle ear cavity.

Surgery

Surgical instruments were soaked overnight in ethanol solution. The surgical field was sterilized with ethanol before creating the skin incision. The bulla was exposed using a retroauricular incision, about 7 mm length. A small hole of approximately 2 mm in diameter was made using a dental drill, and the round window membrane was visualized with an operating microscope at ×40 magnification. When CAP measurements had to be made more than 3 hours after the initial measurement, the wound was temporarily closed with a stitch in the overlying skin. In less than 24 hours, the small hole was found to be closed by fibrous tissues.

Sound System

Asynchronous tone bursts of 4 and 8 kHz (1-ms rise and fall time) and click sounds were given as stimuli at a pulse rate of 20 per second, from 80 dB (re 20 μPa) to the threshold, in 10-dB decrements. The speaker used was a Telephonics TDH-39P, and

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TABLE 1. *Changes in compound action potential threshold after saline*

	24 h (n = 16)	7 d (n = 15)	28 d (n = 10)
Click	7.1	0.5	−1.4
TB 8 kHz	16.2	−3.9	4
TB 4 kHz	12.6	−1.9	4.3

Changes in the CAP threshold were noted in animals treated with saline at 24 hours, 7 days, and 28 days.

A mild elevation in the CAP threshold was found at 24 hours, but the elevation was reversible at 7 and 28 days.

the sound source was placed 10 cm away from the auricle. The free-field sound pressure was monitored and calibrated using a Brüel & Kjær half-inch condenser microphone.

Recording System and CAP Measurement

A 0.08-mm-diameter Teflon-insulated silver wire with an exposed ball tip was carefully placed on the peripheral round window membrane using a micromanipulator. An Ag-AgCl reference electrode was placed in the neck muscles. The CAP responses were averaged 200 times with a Traveler Express ER-22 (Bio-logic Systems Corp, Mundelein, IL, USA.)

Drug Application

After initial CAP measurement, the middle ear cavity was filled with approximately 0.2-ml GV. The volume of the middle ear cavity in the guinea pig is slightly less than 0.2 ml.

Data Analysis

A threshold response was defined as an N1-P1 signal with an amplitude of 10 μV. The change in the sound pressure level in decibels before and after drug application was defined as the change in hearing. The threshold changes before and after drug application were compared. Student's paired *t* test was used to determine the statistical significance of differences.

Bacteriology

The bacteriostatic activity of GV against bacteria commonly isolated from ears in our clinic, namely, methicillin-resistant *Staphylococcus aureus* (MRSA), *P. aeruginosa, S. pneumoniae, Moraxella catarrhalis,* and *Haemophilus influenzae*, was assessed using a disk diffusion assay.

The bacteria were diluted to 10^6 colony forming units (CFU)/ml and cultured on agar plates for 24 hours. GV (50 or 75 μl) was placed on an 8-mm-diameter disk on each cultured plate. After 24 hours, the diameters of the zones of inhibition of bacterial growth were measured.

TABLE 2. *Elevation in compound action potential threshold after 0.5% GV (n = 4)*

	30 min	1 h
Click	0.71	14
TB 8 kHz	4.39	36.6[a]
TB 4 kHz	0.69	28.8[a]

[a] $p < 0.01$.

No ototoxicity was detected in animals treated with 0.5% w/v solution at 30 minutes. However, the same concentration caused a significant elevation in the CAP threshold for 8- and 4-kHz tone bursts at 60 minutes and complete abolishment of CAP at 24 hours.

TABLE 3. *Elevation in compound action potential threshold after 0.13% GV (n = 4)*

	1 h	2 h	3 h	6 h	24 h
Click	−6.67	21.3	38.6[a]	55.8[a]	61.9[a]
TB 8 kHz	21.5[a]	42.5[a]	46.8[a]	69.3[a]	72[a]
TB 4 kHz	0.97	31.5	31.2[a]	49.5[a]	46.7[a]

[a] $p < 0.01$.

A 0.13% w/v solution caused elevation in the CAP threshold for 8 kHz at 1 hour, and a significant elevation in the CAP threshold was measured for clicks and 8- and 4-kHz tone bursts at 3 hours. At 24 hours, the elevation was severe with no sign of recovery.

RESULTS

CAP Changes

The results after saline infusion are shown in Table 1, which was modified from Table 1 of our previous article (1). At 24 hours, the threshold shift was 7.1 dB for the click stimulus, 16.2 dB for 8 kHz, and 12.6 dB for 4 kHz. Changes in response to the click stimulus were not significant, but changes in response to the 8- and 4-kHz tone bursts were significant. However, at 7 and 28 days, the elevated CAP threshold returned to a normal level. Taken together, these results indicate that the change was temporary. It is possible that the change after saline infusion at 24 hours was either conductive or sensorineural or both.

No ototoxicity was detected in animals treated with 0.5% w/v solution at 30 minutes. However, the same concentration caused a significant elevation in the CAP threshold for 8 and 4 kHz tone bursts at 60 minutes and complete abolishment of CAP at 24 hours (Table 2). A 0.13% w/v solution caused elevation in the CAP threshold for 8 kHz at 1 hour, and a significant elevation in the CAP threshold was measured for clicks and 8- and 4-kHz tone bursts at 3 hours. At 24 hours, the elevation was severe with no sign of recovery (Table 3). Thus, a time-dependent elevation in the CAP threshold was found. Upon application of a dilute (0.13% w/v) solution to the round window for 5 minutes followed by washing with saline, a severe time-dependent elevation in the CAP threshold was observed (Table 4).

Bacteriology

Application of a greater volume of GV (50 versus 75 μl) caused increased bacteriostatic activity. Moreover, dilute

TABLE 4. *Elevation in compound action potential threshold after 0.13% GV, for only 5 minutes on the round window membrane (n = 4)*

	3 h	6 h	12 h	24 h
Click	25.8	36.1[a]	41.1[b]	60.1[b]
TB 8 kHz	44.8[b]	59.5[b]	66.8[b]	71.1[b]
TB 4 kHz	22.5	30.9	45.9	60[b]

Upon application of a dilute (0.13% w/v) solution to the round window for 5 minutes followed by washing with saline, a severe time-dependent elevation in the CAP threshold was observed.

[a] $p < 0.05$.

[b] $p < 0.01$.

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TABLE 5. *Bacteriostatic activity for either 50 or 75 μl of gentian violet*

	Full strength (0.5%)		Two fold (0.25%)		Four fold (0.13%)		Eight fold (0.06 %)	
	50 μl	75 μl	50 μl	75 μl	50 μl	75 μl	50 μl	75 μl
P. aeruginosa	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
S. pneumoniae	17	21	18	20	15	20	14	17
M. catarrahalis	24	28	24	27	24	26	23	26
H. influenzae	22	25	21	24	20	23	18	20
MRSA (stock A)	25	27	18	20	14	16	17	19
MRSA (stock B)	24	27	15	18	14	15	16	19

Unit: mm.

The number in the table indicates the diameter of the zones of inhibition of bacterial growth in millimeters. The diameter of the disk was 8 mm.

solutions of GV (0.06–0.13% w/v) were effective against all bacteria tested, with the exception of *P. aeruginosa* (Table 5).

Pathology

Saline control results at 24 hours are shown in Figure 1. A small amount of protein precipitate was seen mostly in the scala vestibuli, but the hair cells, stria vascularis, and ganglion cells appeared to be intact.

The saline control results at 4 weeks are shown in Figure 2. No new bone formation in the middle ear cavity was detected (Fig. 2 upper left). Hair cells, stria vascularis, and spiral ganglion cells appeared normal. Protein precipitate was seen in the scala vestibuli (Fig. 2, upper right).

In animals treated with GV for 30 minutes, no pathology was noted in the organ of Corti, tectorial membrane, stria vascularis, or spiral ganglion cells (Fig. 3). Inflammatory cells were seen in the scala media adjacent to the stria vascularis (Fig. 1, lower left), and a small amount of protein precipitate was seen in the scala vestibuli adjacent to Reissner's membrane (Fig. 3, upper right).

At 24 hours after GV application, marked changes were detected in the scala vestibuli, scala media, and scala tympani, consisting of erythrocytes and massive protein precipitates (Fig. 4, upper left); outer and inner hair cells showed edema. No pathology was found in the stria vascularis or spiral ganglion cells.

At 6 weeks after GV application, hydrops of all cochlear turns was noted (Fig. 5, upper right), and new bone formation in the middle ear cavity was detected (Fig. 5, upper left). The stria vascularis showed marked vacuolation and edema (Fig. 5, lower left), and hair cells were lost (Fig. 5, upper right). In the photo, granulation and new bone formation were apparent in the scala tympani.

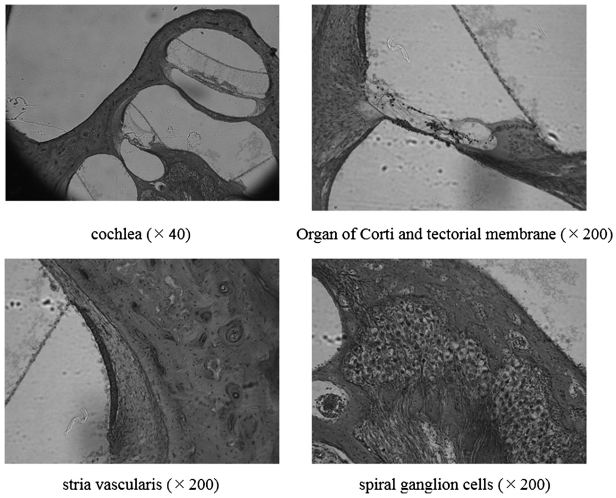

FIG. 2. Histopathologic changes of the temporal bone 4 weeks after saline application.

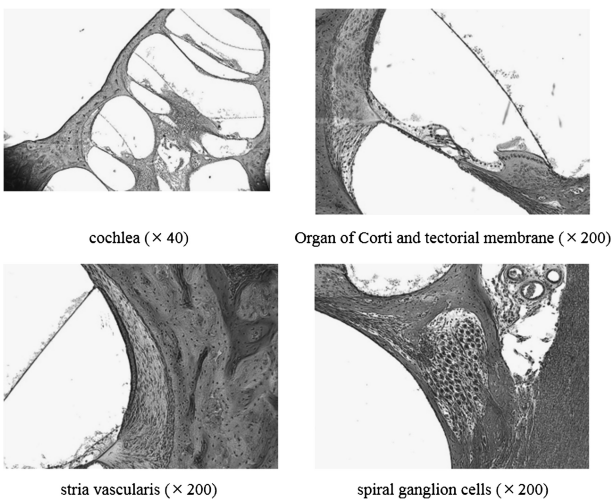

FIG. 1. Histopathologic changes of the temporal bone 24 hours after saline application.

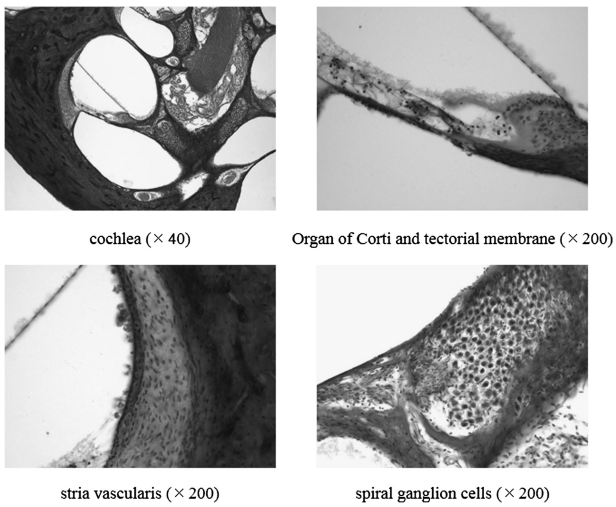

FIG. 3. Histopathologic changes of the temporal bone 30 minutes after GV application.

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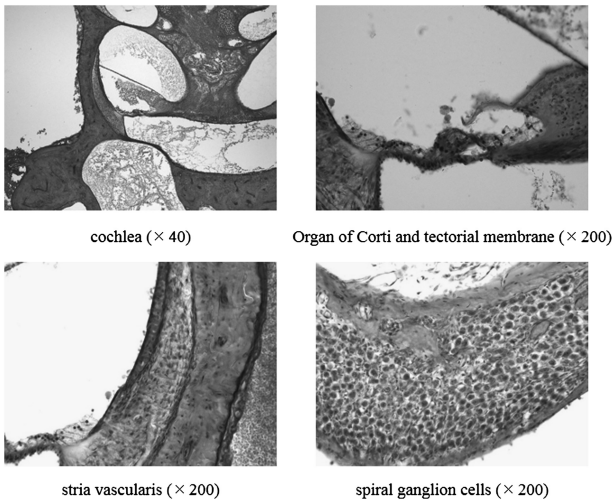

FIG. 4. Histopathologic changes of the temporal bone 24 hours after GV application.

No pathologic changes were noted in the spiral ganglion cells (Fig. 5, lower right).

DISCUSSION

Otic drops are commonly used for chronic otitis media treatment and for prophylaxis after placement of ventilation tubes; therefore, studies of the ototoxicity of topical compounds applied to the middle ear offer important information to clinicians. The potential ototoxicity of solvents and preservatives in eardrops such as propylene glycol has been recognized (2). Antiseptics are used as a vehicle for antibiotics in eardrops or as a preoperative disinfectant of the surgical field. In animal studies, the potential ototoxicities of ethanol (3) and povidone-iodine solutions (4,5) have been reported. Although a thorough review of eardrop ototoxicity was reported by Picketts et al. in 1997 (6), the ototoxicity of GV was not investigated.

GV, or methylrosaniline chloride ($C_{25}H_{30}ClN_3$; MW 408.0), is a purple-colored triphenylmethane antiseptic dye that is effective against some Gram-positive bacteria, particularly Staphylococcus species, and some pathogenic fungi such as Candida species. It is much less active against Gram-negative bacteria and is ineffective against acid-fast bacteria and bacterial spores. Its activity increases as pH increases. GV has been applied topically as a 0.25% to 2.0% aqueous solution or as a cream for the treatment of bacterial and fungal infections, but its application to unbroken skin is now restricted in the United Kingdom because of concerns over carcinogenicity (7). Necrotic skin reactions (8) and oral ulceration (9) have been reported after the use of topical 1% aqueous solutions of GV.

Two studies of the ototoxicity of GV are available. Spandow et al. (10) assessed changes of latencies in auditory brainstem response (ABR) in rats. They applied 1% aqueous solution to the round window membrane and found no change in the ABR at 30 minutes but found increased thresholds to 1 and 6 kHz sounds at 2 hours. Moreover, no reversal of prolonged latency was observed after 1 week. Their result is similar to our finding that 0.5% w/v GV at 30 minutes did not cause changes in CAP, but an increased threshold was noted at 1 hour, with no observed recovery. In addition, we detected a much larger threshold change with 0.5% w/v GV than with 0.13% w/v GV; thus, concentration-dependent ototoxicity was apparent in our study.

Tom (11) reported tilting of the head in 3 of 4 guinea pigs 3 weeks after instillation of 1% GV in the middle ear cavity, indicating strong and instant vestibule-toxicity of GV. Additionally, histopathologic changes were detected in the temporal bone and massive new bone formation in the middle ear cavity. His results agree with our histopathologic findings of massive new bone formation in the middle ear cavity at 6 weeks.

Table 1 shows that saline resulted in a degree of hearing loss at 24 hours, but the hearing returned to normal at 7 and 28 days. The reversible nature of the CAP suggests that the change in the CAP threshold at 24 hours was more likely to be conductive, but some degree of reversible sensorineural components could not be ruled out. At 24 hours, GV resulted in quite severe elevation in the CAP threshold compared with saline. The irreversible elevation of the CAP threshold with GV indicates that the change is sensorineural. It is obvious that the lack of microscopic damage to the hair cells does not necessarily indicate normal inner ear function. Crifo (12,13) studied the ototoxicity of aspirin in the guinea pig using shiver audiometry and observed no appreciable recovery of hearing after 22 days; however, the histologic findings were normal. After the use of GV, gross inflammation of the middle ear mucosa and new bone formation of the middle ear cavity were severe. This inflammation has special clinical significance because severe inflammation

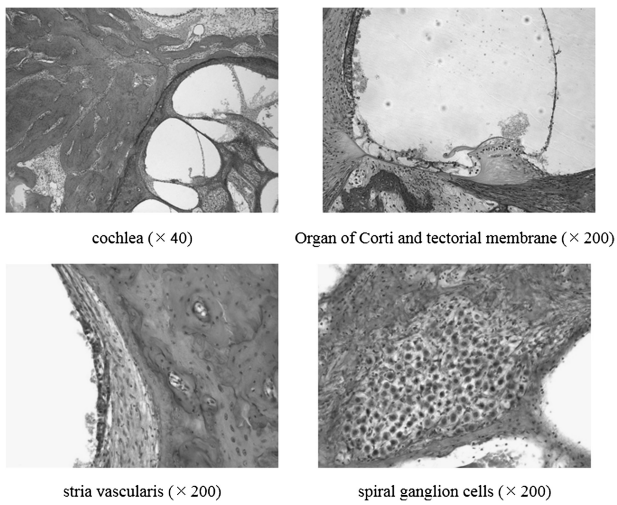

FIG. 5. Histopathologic changes of the temporal bone 6 weeks after GV application.

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and damage to the middle ear mucosa may result in immunosuppression in the mucosa and thus aggravate a bacterial infection already present in the middle ear cavity.

Although GV is effective for treatment of the discharging ear, our data strongly suggest that GV should not be used in the middle ear cavity or in the external canal with perforated tympanic membrane. Upon application of GV to the round window for 5 minutes followed by thorough washing with saline, continuous elevation of the CAP threshold was noted, indicating that GV rapidly entered the inner ear cavity through the round window membrane. The precise amount of GV that penetrated into the inner ear through the round window is not known.

This study used guinea pigs as the animal model of GV ototoxicity. Rodents have a markedly thinner round window membrane than humans; thus, ototoxic effects might be more conspicuous or overestimated in rodents. Another factor that should be kept in mind is that eardrops are generally used in infected ears, which have edematous middle ear mucosa, discharge, and pus on the round window niche, thereby preventing direct contact and penetration of chemicals of the middle ear cavity into the inner ear. The round window membrane in the human ear is thicker in otitis media (14), providing a greater barrier.

Therefore, our intact ear animal model might result in overestimation of the ototoxic effect. Despite the factors mentioned previously, we do not recommend the use of GV in the middle ear cavity. The use of GV should be limited to the external canal only.

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