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Anti-microbial and therapeutic effects of modified Burow's solution on refractory otorrhea

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Abstract

Objective: Burow's solution, which contains 13% aluminum acetate, has been shown to be effective against chronic otitis media. Since the preparation of Burow's solution is time-consuming, its rapid preparation method has been recently developed. In this study, we evaluated the therapeutic effects of the modified Burow's solution on refractory otorrhea in patients with chronic suppurative otitis and its anti-microbial activity *in vitro*.

Methods: Fourteen ears of 12 patients with chronic otitis media, granular myringitis, otitis externa and postoperative mastoid cavity problems were treated topically with cotton swab/ball soaked with modified Burow's solution or its four-fold diluted ear drops once a week. We then examined the antimicrobial spectrum of modified Burow's solution against clinical bacterial isolates from otorrhea and laboratory bacterial strains *in vitro*.

Results: In all ears, refractory otorrhea disappeared after 1–17 weeks treatment of modified Burow's solution with a mean of 5.4 weeks without apparent side-effects such as ototoxicity. Modified Burow's solution inactivated all Gram positive bacteria within 5 min except *Enterococcus* species, all Gram negative bacteria including *Pseudomonas aeruginosa* within 30 s and *Candida albicans* within 2 min. In addition, modified Burow's solution inactivated MRSA completely within 5 min, while 80.6% of MRSA survived even a 20-min contact with 0.3% ofloxacin.

Conclusion: These findings indicate that modified Burow's solution, in addition to bearing a broad antimicrobial activity, is as effective as the original Burow's solution in the treatment of chronic suppurative otitis.

Keywords: Burow's solution; Aluminum acetate; Otorrhea; Chronic suppurative otitis; Bactericidal effect

1. Introduction

Burow's solution, which contains 13% aluminum acetate (pH 3), had been invented for the treatment of chronic suppurative otitis media by a German surgeon, Karl August von Burow in the late 19th century [1]. However, after the introduction of antibiotics into the treatment of suppurative otitis, the usefulness of Burow's solution was markedly limited. In 1998, Thorp et al. revisited the clinical efficacy of Burow's solution on chronic suppurative otitis media and showed its high improving rate and its anti-microbial activity against common responsible bacteria in discharging ear [2–4]. Then, Terayama et al. reported the therapeutic effects of Burow's solution on refractory suppurative otorrhea in opened mastoid cavity infections and otomycosis, indicating the

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Table 1
Therapeutic effects of modified Burow's solution on refractory otorrhea in patients with chronic suppurative otitis.

Patient	Gender[a]	Age	Ear	Diagnosis[b]	Duration of illness (months)	Microbiological examination[c]	RBS treatment[d]	Times for remission[e]	Audiogram (before/after)[f]
1	F	77	R	COM	53	PA	Cotton ball	1	68.8/70.0
			L	OE, GM	38	PA, MRSE	Cotton ball	5	96.3/65.0
2	F	70	R	COM	120	MSSA, α-ST, γ-ST	Cotton swab	2	65.0/58.8
3	M	69	R	GM	4	α-ST	Cotton swab	3	51.2/50.0
4	M	16	L	OE, GM	24	MSSA	Cotton ball	1	43.8/28.8
5	M	68	R	COM	5	CN	Ear drop	2	78.8/37.5
6	F	66	R	GM	60	EC, MSSA	Cotton ball	9	40.0/38.8
7	F	80	L	OE, GM	24	MRSA	Ear drop	12	–
8	M	76	L	PMCP	7	PA	Cotton ball	2	–
9	F	85	L	COM	29	PV, CN	Ear drop	2	–
10	F	82	R	OE, GM	5	PM, CN	Cotton swab	11	–
			L	OE, GM	6	PA, PV	Cotton swab	17	–
11	M	93	R	COM	4	MRSA, CN	Cotton swab	4	–
12	F	92	L	GM	38	EN, CN	Cotton ball	5	–

[a] F: female, M: male.
[b] COM: chronic otitis media, GM: granular myringitis, OE: otitis externa, PMCP: postoperative mastoid cavity problems.
[c] AM: *Aeromonas* species, BU: *Burkholderia cepacia*; BC: *Bacillus species*, CF: *Citrobacter freundii*, CN: *Corynebacterium* species, EC: *Enterobacter cloacae*, EN: *Enterococcus* species, KP: *Klebsiella pneumoniae*, MRSA: methicillin-resistant *Staphylococcus aureus*, MRSE: methicillin-resistant *Staphylococcus epidermidis*, MSSA: methicillin-sensitive *S. aureus*, PA: *Pseudomonas aeruginosa*, PM: *Proteus mirabilis*, PV: *Providencia* species, α-ST: α-hemolytic *Streptococcus*, β-ST: β-hemolytic *Streptococcus*, γ-ST: γ-hemolytic *Streptococcus*.
[d] Cotton ball/swab: treatment with cotton ball/swab soaked with modified Burow's solution; ear drop: treatment with 0.1 ml of 4-fold diluted modified Burow's solution.
[e] Topical treatment of modified Burow's solution was performed once a week.
[f] Not tested.

usefulness of Burow's solution in the treatment of chronic ear infection [5,6].

However, the original preparation method of Burow's solution is time-consuming and requires at least four days to finalize. Recently, Ishibashi et al. developed a modified method for preparing Burow's solution, which takes only a few hours and showed that this solution has anti-bacterial activity *in vitro* against *Staphylococcus aureus*, including methicillin-resistant strains (MRSA) that is equivalent to that of the original Burow's solution [7].

In this study, we evaluated the therapeutic effects of modified Burow's solution on refractory otorrhea in patients with chronic otitis media, granular myringitis, otitis externa and postoperative mastoid cavity problems. We then examined the anti-microbial spectrum of modified Burow's solution *in vitro*. We concluded that topical modified Burrow's solution is effective in the treatment of refractory ear suppurations.

2. Materials and methods

2.1. Patients

Twelve patients with chronic otitis media, granular myringitis, otitis externa and postoperative mastoid cavity problems (seven males and five females; ages ranging from 16 to 93 years old; mean age: 72.8 years) were enrolled in this study (Table 1). The patients suffered from chronic otorrhea, which was refractory to toileting of ear canal with saline and/or 0.3% ofloxacin (Taribid®, Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan) ear drops for 4–120 months with a mean period of 29.8 months. Microbiological examinations of the otorrhea were performed prior to the treatment of modified Burow's solution, and pure-tone audiograms were taken in seven ears after treatment to compare it with pre-treatment audiograms for the evaluation of its ototoxicity.

2.2. Modified Burow's solution

Modified Burow's solution (13% aluminum acetate, pH 3) was prepared according to the protocol reported by Ishibashi et al. [7]. The compounding information of the modified Burow's solution is shown in Table 2. Namely, 12.1 g of aluminum acetate ($Al_2O(CH_3COO)_4$), 25 ml of acetic acid (33%), 4.5 g of tartaric acid and 75 ml of purified

Table 2
Compounding of modified Burow's solution.

Ingredient	Amount
Aluminum acetate ($Al_2O(CH_3COO)_4$)	12.1 g
Acetic acid (33%)	25 ml
Tartaric acid	4.5 g
Purified water	75 ml
Total	100 ml

The above 4 ingredients are mixed, suspended and dissolved completely by heating at 80 °C for 2 h. Because 5 ml will be evaporated, more sterile purified water is added for a total amount of 100 ml after the liquid is passed through a sterile filter.

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water are mixed, suspended and dissolved completely by heating at 80 °C for 2 h. Because 5 ml will be evaporated, more sterile purified water is added for a total amount of 100 ml after the liquid is passed through a sterile filter. Modified Burow's solution has anti-bacterial activity *in vitro* against *S. aureus*, including MRSA that is equivalent to that of the original Burow's solution (13% aluminum acetate, pH 3) [7]. Fourteen ears of 12 patients were treated topically with cotton swab/ball soaked with modified Burow's solution or its four-fold diluted ear drops. One minute later, the cotton swab/ball soaked with modified Burow's solution was removed or four-fold diluted modified Burow's solution was washed out with 10 ml of warm sterile saline. All patients were treated once a week at our outpatient clinic under medical supervision for its safe use. In the initial treatment phase, half of patients complained of short-lasting ear pain.

2.3. Anti-microbial assay

Anti-microbial spectrum of modified Burow's solution against clinical bacterial isolates from otorrhea and laboratory bacterial strains was examined *in vitro*. Microorganisms used in this study were five Gram positive bacteria including methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), *Enterococcus*, *Corynebacterium* and *Bacillus*, seven gram negative bacteria including *Pseudomonas aeruginosa* (PA), *Escherichia coli*, *Proteus mirabilis*, *Aeromonas*, *Providencia*, *Burkholderia cepacia* and *Klebsiella pneumonia*, and a fungus, *Candida albicans*.

Bacterial and fungal strains were grown on agar plates at 37 °C for 1–2 days. The colonies were then suspended in sterilized saline and the turbidities of the suspensions were equivalent to MacFaland 1.0 [10^8–10^9 colony-forming units (CFU)/ml]. A part of each microbial suspension (50 μl) was inoculated into 5 ml each of modified Burow's solution (13% aluminum acetate) or 0.3% ofloxacin (Taribid®, Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan), and the mixtures were then incubated at room temperature. Sterilized saline was used as control. Samples (0.1 ml) were collected from the mixtures at 0.5, 2, 5, 10, 20 and 40 min after inoculation. The serial 100-fold dilutions (10^{-2}, 10^{-4} and 10^{-6}) of the samples were immediately prepared and 0.2 ml of each dilution was spread on duplicated agar plates. The plates were firstly incubated at 30 °C for 48 h and then stored for additional 5 days at room temperature. The number of colonies formed on the agar plates was counted everyday for 7 days. The mean viable cell number in each sample was calculated from the colony counts on the plates when a colony number reached a plateau. The percentages of the surviving test microorganisms were calculated by dividing the viable cell number in each sample with that in control solution (sterilized saline), and the results were expressed as mean values of two independent experiments.

3. Results

Fourteen ears of 12 patients with chronic suppurative otitis media, granular myringitis, otitis externa and postoperative mastoid cavity problems were treated topically with cotton swab/ball soaked with modified Burow's solution or its four-fold diluted ear drops once a week. In all ears, refractory otorrhea disappeared after 1–17 weeks treatment of modified Burow's solution with a mean of 5.4 weeks (Table 1). In 10 (71%) of the 14 ears, remission was obtained within 5 weeks treatment with modified Burow's solution. Even in the two ears infected with MRSA, remission was obtained after 4 and 12 weeks of this treatment. In four ears infected with PA, remission was obtained after 1, 2, 5 and 17 weeks. On the other hand, in seven ears in six patients treated with topical modified Burow's solution, the hearing levels either recovered ($n = 3$) or remained ($n = 4$) (Table 1).

The bactericidal potential of modified Burow's solution was examined using microbial isolates from the patients and laboratory microbial strains *in vitro*. Accordingly, modified Burow's solution inactivated all Gram positive bacteria within 5 min except for *Enterococcus* species. It also inactivated all Gram negative bacteria including PA within 30 s and a fungus, *C. albicans,* within 2 min (Table 3).

The bactericidal effect of modified Burow's solution on MSSA was similar to that of ofloxacin, which inactivated the strain within 5 min. But, modified Burow's solution inactivated MRSA within 2 min, while 80.6% of MRSA survived a 20 min contact with ofloxacin (Table 4).

4. Discussion

Chronic otologic infections are sometimes refractory to treatment, despite appropriate systemic and topical antibiotics. Recently, clinical efficacy of Burow's solution on chronic suppurative otitis media was re-evaluated and the remission rate was reported to reach 80% in all of the cases [2–4]. Burow's solution was also shown to be effective on other chronic suppurative otitis including otitis externa, granular myringitis and postoperative mastoid cavity problems [5,6]. In the present study, we used modified Burow's solution prepared according to a rapid method and administered topically to treat refractory otorrhea in patients with chronic otitis media, granular myringitis, otitis externa, and postoperative mastoid cavity problems. In most ears (71%), remission was obtained within 5 weeks treatment. These findings indicate that modified Burow's solution is as effective as the original Burow's solution in the treatment of chronic suppurative otitis.

Clayton et al. preformed a double-blinded, randomized, prospective trial and demonstrated that otorrhea improved in 67% of ears treated with topical 8% aluminum acetate antiseptics and in 68% of those treated with topical gentamicin sulphate in patients with otitis externa, chronic

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Table 3
Anti-microbial activity of modified Burow's solution against clinical bacterial isolates from otorrhoea and laboratory bacterial strains.

Test strains[a]	Survival rate (%)[b]						
	0 min	0.5 min	2 min	5 min	10 min	20 min	40 min
Gram positive bacteria							
MSSA	100	77.5	0				
MRSA	100	97.9	58.0	0			
Enterococcus sp.	100	93.7	93.4	93.2	78.1	47.4	0
Corynebacterium sp.	100	55.2	0				
Bacillus sp.	100	0					
Gram negative bacteria							
Pseudomonas aeruginosa	100	0					
Escherichia coli	100	0					
Aeromonas sp.	100	0					
Providencia sp.	100	0					
Proteus mirabilis	100	0					
Burkholderia cepacia	100	0					
Klebsiella pneumoniae	100	0					
Fungus							
Candida albicans	100	55.3	0				

[a] Stocked laboratory strains used were MRSA, *P. aeruginosa*, *E. coli*, and *C. albicans*. Other strains were clinical isolates from ear discharges obtained in this study.
[b] The percentages of the surviving test microorganisms were calculated by dividing the viable cell number in each sample with that in control solution (sterilized saline). The results are expressed as mean values of two independent experiments.

otitis media and postoperative mastoid cavity problems [8]. It is, therefore, suggested that 13% aluminum acetate modified Burow's solution has anti-bacterial effect similar to that of aminoglycosides.

In the present study, modified Burow's solution inactivated all Gram positive bacteria except *Enterococcus* species within 5 min, and all Gram negative bacteria including PA within 30 s and a fungus, *C. albicans* within 2 min. These findings further indicate that modified Burow's solution has a broad spectrum anti-microbial activity similar to that of the original Burow's solution.

Enterococcus species survived in modified Burow's solution for 20 min, probably because of their resistance to acidic environments [9]. Indeed, the anti-bacterial effects of Burow's solution have been considered to be due to its acidity [1], and various concentrations of acetic acid solution have been used as topical treatment for granular myringitis and chronic suppurative otitis media [10,11].

Moreover, *in vitro* studies showed a similarity in anti-bacterial activity between the more acidic acetic acid and Burow's solution, suggesting that additional anti-bacterial effects of the latter solution are due to its aluminum component [2]. This observation is in line with the finding of Ishibashi et al. who demonstrated that the anti-bacterial potency of Burow's solution was dependent on its concentration of aluminum [7]. In fact, aluminum is a toxic agent, which has been shown to alter the molecular structure of cell membrane, resulting in changes in its physiological properties [12]. Taken together, these observations suggest that the anti-microbial effects of Burow's solution are due to the combination of its acidity and aluminum component.

Clinically, infection with MRSA has been increasing in Japan. MRSA was frequently detected in patients with chronic suppurative otitis, especially those who had a previous antibiotics therapy [13] and whose otorrhea became refractory to aural toileting, and povidone–iodine [14]. In the present study, remission was obtained in two ears infected with MRSA 4 and 12 weeks after this treatment. Moreover, modified Burow's solution inactivated MRSA completely within 5 min, while 80.6% of MRSA survived even after a 20-min contact with 0.3% ofloxacin *in vitro*. These findings suggest that topical application of modified

Table 4
Bactericidal activities of modified Burow's solution and ofloxacin against MSSA and MRSA.

Test solution[a]	Survival rate (%)[b]											
	MSSA						MRSA					
	0 min	0.5 min	2 min	5 min	10 min	20 min	0 min	0.5 min	2 min	5 min	10 min	20 min
Burow	100	97.9	58.0	0	0	0	100	77.5	0	0	0	0
OFLX	100	98.2	83.0	0	0	0	100	98	90	80.6	80.6	80.6

[a] Burow: modified Burow's solution, OFLX: 0.3% ofloxacin.
[b] The percentages of the surviving test microorganisms were calculated by dividing the viable cell number in each sample with that in control solution (sterilized saline). The results are expressed as mean values of two independent experiments.

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Burow's solution is useful for the treatment of refractory discharging ear infected with MRSA.

It is possible that long-term use of modified Burow's solution in place of antibiotics will decrease the risk of colonization by antibiotics-resistant microorganisms such as MRSA and drug-resistant PA. The four main mechanisms underlying bacterial acquisition of resistance to antibiotics are drug inactivation or modification, alteration of target site, alteration of metabolic pathway and reduced drug accumulation [15]. Burow's solution that contains 13% aluminum acetate acts on the bacterial surface membrane without specific target molecules, and is neither inactivated nor metabolized by microorganisms.

In the present study, seven ears were tested by pure-tone audiometry and their hearing levels were either recovered or remained after the treatment with cotton swab/ball soaked with modified Burow's solution or its four-fold diluted ear drops. However, Oishi et al. reported two cases showing the potential ototoxicity of Burow's solution [16]. In a previous animal study, the application of ear drops containing 2% acetic acid at pH 2 to the round window decreased the endolymphatic potential and lowered the pH of perilymph, whereas application of acidic perilymph at pH 4 induced no significant changes [17]. These findings suggest that the round window membrane is a key route of ototoxic agents to the cochlea and that the acidity of Burow's solution (pH 3) is associated with its ototoxicity. Although Burow's solution is generally used as ear drop [3,5,6], we used cotton balls or swabs soaked with it to avoid its direct application to the round window membrane in some patients with chronic otitis media. For the future, Burow's solution should not be used in the form of ear drop in patients with a perforated tympanic membrane, even though it is diluted four-fold, because its acidity (pH 3.5) has potential ototoxicity [3].

In conclusion, modified Burow's solution not only can be prepared in a short time, but is also as effective in the treatment of chronic suppurative otitis as the original Burow's solution. In addition, modified Burow's solution has a broad spectrum anti-microbial activity, including MRSA *in vitro*. These findings suggest that topical application of modified Burow's solution is effective in the treatment of refractory otorrhea in patients with chronic suppurative otitis.

Conflict of interest

The authors declare that they have no conflicts of interest.

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