ORIGINAL ARTICLE

The Efficacy of Boric Acid in Otomycosis: An in Vitro Study

Aydın Karaarslan, M.D., K. Murat Özcan, M.D., Müge Özcan, M.D.

From the Department of Microbiology, Medicine Faculty of Ankara University (A. Karaaslan); Departments of Otolaryngology the 4th and 1st, Ankara Numune Education and Research Hospital (K. M. Özcan, M. Ozcan), Ankara, Turkey.

Correspondence:
K. Murat Özcan, M.D.
Yücetepe Sitesi, A Blok,
No: 59/6, 06580 Anıttepe,
Ankara, Turkey.

Tel: +90 312 473 01 87
Fax: +90 312 310 34 60
e-mail: kursatmuratozcan@yahoo.com

Submitted: January 22, 2005
Accepted: April 3, 2004

Mediterr J Otol 2005;2:00-00.

OBJECTIVES: Topical antiseptics and antifungal agents are frequently used for the treatment of otomycosis. Topical use of boric acid solution in alcohol is also recommended, and some studies report its clinical efficacy. However, there are no studies in the English literature concerning the *in vitro* efficacy of boric acid salts on fungi isolated from the ears with otomycosis. In this study, we investigated the *in vitro* effectiveness of boric acid solutions in distilled water and in ethanol on *Aspergillus* species and *Candida albicans* that were isolated from the ears with otomycosis.

MATERIALS AND METHODS: *In vitro* susceptibilities of *Aspergillus* species including *A. niger, A. fumigatus, A. flavus, A. terreus* and *C. albicans* strains to boric acid solution in distilled water (eau boric), boric acid solution in 70% ethanol (alcohol boric), and to 70% ethanol were investigated using the agar dilution method described by Shubair and Larsen. Concentrations of the media ranged from 0.25% to 4%.

RESULTS: Eau boric and alcohol boric were equally effective to inhibit the growth of *C. albicans* in all concentrations equal to or greater than 0.5% ($p>0.05$). However, at 0.25%, both solutions were equally ineffective to inhibit the growth of the *Aspergillus* species ($p>0.05$). At 0.5% concentration, alcohol boric was found significantly more effective against all *Aspergillus* species compared to eau boric ($p<0.001$). The inhibition of *A. niger* was significantly more prominent with 1% alcohol boric compared to 1% eau boric ($p<0.001$). Both solutions were equally effective against all fungi at the concentrations of 2% and 4% ($p> 0.05$). When compared to alcohol boric and eau boric, ethanol was less effective to inhibit the growth of all fungi at all concentrations ($p<0.001$).

CONCLUSION: The results of this *in vitro* study suggest that both alcohol boric and eau boric inhibit the growth of *Aspergillus* species and *C. albicans* isolated from the ears with otomycosis. Alcohol boric produces maximal inhibition in lower concentrations compared to eau boric; thus, it may be considered a cost-effective treatment option for otomycosis.

Otomycosis is the fungal infection of the ear, usually involving the external auditory meatus.[1] *Aspergillus* and *Candida* species are the most common offending agents.[2,3] Meticulous aspiration of the ear canal is important for the treatment. Topical antifungals and antiseptics are also frequently used.

Topical use of boric acid solution in alcohol is recommended for the treatment of otomycosis[4] and some studies report its clinical efficacy.[5-7] However, there are no studies in English literature concerning *in vitro* inhibitory effect of boric acid salts on fungi isolated from the ears with otomycosis.

In this study, we investigated the *in vitro* inhibitory effects of boric acid solutions in distilled water and in ethanol on *Aspergillus* species and *Candida albicans* that were isolated from the ears with otomycosis.

MATERIALS AND METHODS

In vitro susceptibilities of 55 *Aspergillus* species (29 *A. niger*, 14 *A. fumigatus*, 10 *A. flavus*, 2 *A. terreus strains*) and 7 *C. albicans* strains to boric acid solution in distilled water, boric acid solution in 70% ethanol, and to 70% ethanol were investigated using the agar dilution method described by Shubair and Larsen.[8] Boric acid solution in distilled water will be termed as "eau boric", boric acid solution in 70% ethanol as "alcohol boric", and 70% ethanol as "ethanol" in the rest of the text for convenience.

The fungal strains tested were isolated from patients with otomycosis and identified by conventional mycological methods.[9]

Three different Sabouraud dextrose agar media containing eau boric, alcohol boric or ethanol with final concentrations ranging from 0.25 to 4% were prepared in tubes with lid.

Aspergillus colonies on Sabouraud dextrose agar were covered with 1 ml of sterile 0.85% saline and scraped with a sterile pipette. The suspensions were transferred into sterile tubes. The suspensions of *Candida albicans* were also prepared in sterile saline. The turbidity of fungal suspensions was measured by spectrophotometry at 530 nm and adjusted to obtain a final inoculum of $1x10^6$ - $5x10^6$ CFU/ml. Ten microliters of these suspensions were poured into the Sabouraud dextrose agar tubes to acquire the final inocula ranging from $1x10^4$ - $5x10^4$ cells and were incubated at 35 °C for 48 hours. The presence or absence of growth was evaluated as a percentage.

The chi-square test was used for statistical analysis and a *p* value of less than 0.05 was regarded as statistically significant.

RESULTS

Eau boric completely inhibited the growth of *C. albicans* in the concentration of 0.5%. The growth of *A. terreus* was inhibited at 1%, whereas 2% eau boric was required to inhibit the growth of other *Aspergillus* species (Table 1).

Alcohol boric inhibited the growth of *C. albicans* and all *Aspergillus* species in a concentration of 0.5% (Table 1).

Seventy percent ethanol alone, did not completely inhibit the growth of either *Aspergillus species* or *C. albicans* even in a concentration of 4% (Table 1).

Statistically, it was evident that both eau boric and alcohol boric were equally effective to inhibit the growth of *C. albicans* in all concentrations equal to or greater than 0.5% ($p>0.05$). However, at 0.25%, both solutions were equally ineffective to inhibit the growth of the *Aspergillus* species ($p>0.05$). At 0.5% concentration, alcohol boric was found significantly more effective against all *Aspergillus* species compared to eau boric ($p<0.001$). The inhibition of *A. niger* was significantly more prominent with 1% alcohol boric compared to 1% eau boric ($p<0.001$). Both solutions were equally effective against all fungi at the concentrations of 2% and 4% ($p> 0.05$).

When compared to alcohol boric and eau boric, ethanol was less effective to inhibit the growth of all fungi at all concentrations ($p<0.001$).

DISCUSSION

The results of this study suggest that both alcohol boric and eau boric are effective to inhibit the growth of *C. albicans* and *Aspergillus* species *in vitro*. The inhibitory effect of alcohol boric is evident in lower concentrations compared to those of eau boric.

Topical antifungals and antiseptics are commonly used for the treatment of otomycosis. The topical antifungals are marketed as dermatological preparations for skin or nail, however, otic drop forms are not yet available in our country as well as many others. This may be due to the lack of studies on the ototoxicity of these compounds. In addition, they are expensive compared to antiseptic solutions. In

Table 1. The growth rates of *Aspergillus* species and *Candida albicans* in Sabouraud dextrose agar media containing eau boric, alcohol boric, and 70% ethanol with final concentrations ranging from 0.25 to 4%

	Concentration (%)	*A. niger* (%)	*A. fumigatus* (%)	*A. flavus* (%)	*A. terreus* (%)	*C. albicans* (%)
Eau boric	0.25	100	100	100	100	28.57
	0.5	100	100	100	100	0
	1	20.68	14.28	10	0	0
	2	0	0	0	0	0
	4	0	0	0	0	0
Alcohol boric	0.25	100	100	100	100	42.85
	0.5	0	0	0	0	0
	1	0	0	0	0	0
	2	0	0	0	0	0
	4	0	0	0	0	0
70% ethanol	0.25	100	100	100	100	100
	0.5	100	100	100	100	100
	1	100	100	100	100	100
	2	100	100	100	100	100
	4	93.1	100	70	100	100

Turkey, the two-week treatment cost of otomycosis with tioconazole cream is almost ten times as high as the treatment cost with alcohol boric.

Bassiouny et al.[10] studied *in vitro* efficacies of clotrimazole, econazole, miconazole, and cyclopirox-olamine on fungi responsible for otomycosis and concluded that clotrimazole and econazole were more effective. Stern et al.[11] reported similar results.

Jain and Agrawal[12] investigated the inhibitory effects of volatile substances such as ammonia, carbon disulphide, and acetic acid on fungi responsible for otomycosis and reported that all inhibited mycelial growth and sporulation, ammonia being the most potent.

Boric acid solutions are commonly used in Turkey for the treatment of bacterial and fungal infections of the ear. Ozcan et al.[7] reported complete clinical recovery of otomycosis in 77% with the topical use of 4% alcohol boric and frequent suction cleaning of the ear canal. Erkan et al.[5] reported complete resolution in three weeks with the use of boric acid salt in ethanol. Than et al.[6] suggested that the use of boric acid salt was a good alternative to topical 5-fluorouracil and nystatin.

The efficacy of boric acid on vaginal candidiasis was demonstrated both *in vivo* and *in vitro*.[8,13] Otero et al.[13] investigated the efficacy of boric acid on *C. albicans* and *C. glabrata* isolated from vaginal candidiasis. They reported that 0.4% boric acid inhibited the growth of 97.2% of the *Candida* species.[13] Shubair and Larsen[8] reported total inhibition of the growth of *C. albicans* at the same concentration.The agar dilution method was used in both studies.

To our knowledge, this is the first report in the English literature investigating the *in vitro* efficacy of boric acid salts on *Aspergillus* species and *C. albicans* isolated from the ears with otomycosis.

We employed the agar dilution method as described by Shubair and Larsen[8] and Otero et al.[13] The use of ethanol alone produced minimal inhibition of the growth of the fungi in 4% concentration. Both eau boric and alcohol boric produced total inhibition of the mycelial growth, however, the inhibitory effect of the latter was evident in lower concentrations.

The results of this *in vitro* study suggest that both alcohol boric and eau boric inhibit the growth of *Aspergillus* species and *C. albicans* isolated from the ears with otomycosis. Alcohol boric produces maximal inhibition in lower concentrations compared to eau boric; thus, it may be considered a cost-effective treatment option for otomycosis.

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