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Comparative study of epidermal growth factor and observation only on human subacute tympanic membrane perforation☆,☆☆

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ABSTRACT

Objective: To compare the effects of epidermal growth factor (EGF) and observation only on human subacute tympanic membrane perforation (TMP).
Methods: A total of 44 patients with traumatic TMPs > 2 months after trauma were divided into an observation group (n = 18) and EGF group (n = 26). Patients in the EGF group underwent direct application of EGF without stripping of the perforation edge. All patients were followed up for at least 6 months. The TMP closure rate, closure time, and hearing gain were evaluated.
Results: At 6 months, 25 of 26 (96.2%) perforations achieved complete closure with a mean closure time of 9.1 ± 3.9 days (range, 3–14 days) in the EGF group. However, only 11 of 18 (61.1%) perforations achieved complete closure in the observation group, with a mean closure time of 20.6 ± 10.7 days (range = 9–71 days). The patients in the EGF-treated group had significantly improved closure rates (P = 0.026) and a reduced closure time (P < 0.01) compared to those in the observation group. The difference in mean hearing improvement between the two groups was not statistically significant (P = 0.86).
Conclusions: Topical application of EGF improved the closure rate and shortened the closure time of human subacute TMPs compared with spontaneous healing, the stripping of the perforation edge was unnecessary. This treatment is simple and convenient and should be recommended pre-myringoplasty.

1. Introduction

Most of traumatic tympanic membrane perforations (TMPs) tend to heal spontaneously within 4–8 weeks, a few TMPs failed to or delayed healing although the perforations had lasted > 2 months, thereby formed subacute perforations and affected the patient's living quality. Some scholars suggested that myringoplasty should be performed if a perforation fails to heal within 3 to 6 months [1,2]. Epidermal growth factor (EGF), a 53-amino-acid mitogenic polypeptide present in many mammalian species, is being investigated for its potential to expedite TMP healing. EGF stimulates keratinocyte division *in vitro* and epidermal regeneration *in vivo* [3,4]. EGF has no apparent risk for ototoxicity or complications when applied topically to the external and middle ears of animals and humans [5–7]. The effects of EGF on acute TMPs have been reported [5–10]. Our recent clinical studies showed that although the topical application of EGF did not improve the closure rate of acute TMPs compared to spontaneous healing, it significantly reduced the closure time [5–8]. However, the effects of EGF on traumatic subacute TMPs were unclear. We investigated the effects of EGF without stripping the perforation edge of traumatic subacute TMPs in this study.

2. Materials and methods

This study was approved by the Institutional Ethical Review Board of Wenzhou Medical College-Affiliated Yiwu Hospital in Yiwu, Zhejiang, China. Informed consent was obtained from all participants.

Subjects were recruited from among consecutive patients diagnosed

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Table 1
Demographic data of EGF and observation groups.

	EGF group	Observation group	P value
No.	26	18	–
Sex, (M:F)	7:19	5:13	0.411[a]
Side, (R:L)	6:20	3:15	0.082[a]
Cause of perforation, (SL:B)	24:2	17:1	0.233[a]
Myringosclerosis, (N:Y)	21:5	14:4	0.358[a]
The position of perforation (anteroinferior:posteroinferior)	22:4	15:3	0.09[a]
Age, year	30.1 ± 11.04	31.6 ± 9.83	0.451[b]
Hearing levels, dB	27.6 ± 4.1	25.8 ± 6.7	0.725[b]
Duration of perforation, day	120.3 ± 28.8	119.4 ± 27.3	0.211[b]

$P < 0.05$ was considered statistically significant.
SL: slap injury; B: blast injury; N: no; Y: yes.
[a] χ^2 test.
[b] *t*-Test.

with traumatic TMP who visited the Department of Otorhinolaryngology, Head and Neck Surgery, at Wenzhou Medical College-Affiliated Yiwu Hospital from January 2013 to October 2017. The inclusion criteria were as follows: (1) traumatic TMPs; (2) a period of > 2 months since trauma; and (3) no middle ear infection at the time of the hospital visit. Patients with the infection, or granulation tissue in the middle ear at the time of the first visit were excluded. Age, sex, date of injury, side of the injury, cause of traumatic injury, crust circumferentially around the perforation, and size of the TMP were recorded at the time of the visit.

All patients were examined with an endoscope after cleaning cerumen or blood clots from the external auditory canal (EAC) using a cotton bud soaked in povidone iodine solution, and the site and size of the TMP were documented. The perforation size was divided into medium (1/8–1/4 of the eardrum) and large (> 1/4 of the eardrum) [7]. Standard pure-tone audiometric testing was performed at the initial and final visits or 6 months after treatment. Pure-tone averages were determined for air and bone conduction at 500, 1000, 2000, and 4000 Hz. Subacute traumatic TMP was defined as a TMP that had not closed completely at 2 months post-injury. The clinician described the treatment methods and their advantages and disadvantages to the patients on their first visit. The patients were then randomized into two groups: EGF treatment and observation only. Randomization was performed using a table of random numbers with SPSS software (ver. 11.0 for Windows; SPSS Inc., Chicago, IL).

2.1. Observation group

The EAC was cleaned using a cotton bud soaked in a povidone iodine solution. These patients received no further treatment other than keeping the ear dry.

2.2. EGF group

The EAC was cleaned using a cotton bud soaked in povidone iodine solution. Approximately 0.1 to 0.15 mL (two to three drops) of recombinant bovine EGF solution (21,000 IU/5 mL; Yi Sheng) was applied to the eardrum along the EAC once daily to keep the eardrum moist; the margin of the perforation was not trimmed and no scaffolding material was used.

Follow-up was scheduled twice per week within 1 month after the initial hospital visit and then once per week until complete closure of the perforation, or up to 6 months. In the EGF group, the first application of EGF drops was carried out by a physician. Subsequently, the drops were applied by the patients themselves at home, as instructed, until complete perforation closure was confirmed by a physician. The physician determined whether the patients had correctly applied the EGF drops and whether purulent otorrhea developed during the 2 to 3 days following the start of treatment. The eardrop dose was adjusted as needed to keep the eardrum moist (not excessively wet or dry). Any incorrect application was corrected. The patient was advised to discontinue eardrops if the ear symptoms disappeared or purulent otorrhea developed. Oral amoxicillin and ofloxacin eardrops were administered simultaneously if purulent otorrhea developed. To reduce clinician bias, tympanic membrane closure and otorrhea were documented endoscopically using color photographs at each follow-up visit. The calculation of mean closure time included only those TMPs with complete closure; it did not include those TMPs that failed to close during the follow-up period.

2.3. Statistical methods

The results are expressed as means ± SD or as percentages. Statistical analyses were performed using SPSS software (ver. 11.0 for Windows; SPSS Inc.), and the paired chi-square test and *t*-test were used to compare the closure rate and closure time, respectively. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

3. Results

A total of 44 patients (12 males, 32 females) met the inclusion criteria; their average age was 30.1 ± 6.4 years (range, 17–56 years). All of the 44 patients had medium-sized perforations (18 in the observation group and 26 in the EGF group). Of the 44 patients, 36 patients showed closure of some degree during the first two months, while 8 patients only showed the crust of perforation edge. The mean duration was 119.4 ± 27.3 days in the observation group and 120.3 ± 28.8 days in the EGF group. 41 perforations were caused by a slap or fist to the ear while 3 perforations in blast injury. The age and sex of the patients, injury cause, injury duration, injured side, position of the perforation, preexisting myringosclerosis, and hearing levels were matched between the groups ($P > 0.05$) (Table 1).

The healing outcome and hearing gain at 6 months are shown in Table 2. In the observation group, 11 of 18 (61.1%) TMPs healed spontaneously with a mean closure time of 20.6 ± 10.7 days (range = 9–71 days). However, in the EGF group, 25 of 26 (96.2%) perforations achieved complete closure (Figs. 1 and 2) at a mean of

Table 2
The healing outcome of EGF and observation groups.

	EGF group	Observation group	P value
No	26	18	
Closure rate, %	96.2	61.1	0.026[a]
Mean closure time, days	9.1 ± 3.9	20.6 ± 10.7	< 0.01[b]
Hearing gain, dB	12.1 ± 6.2	11.7 ± 4.8	0.86[b]

[a] χ^2 test.
[b] *t*-Test.

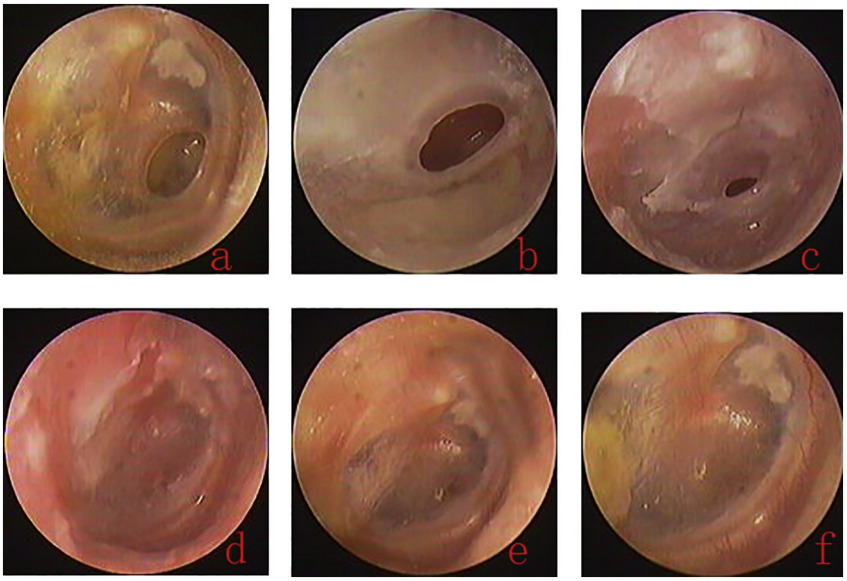

Fig. 1. The healing of medium-sized perforations after EGF treatment: 7 months after perforation (a), 3 days after treatment (b), 10 days after treatment (c), 2 weeks after treatment (d), 5 weeks after treatment (e), and 6 weeks after treatment (f).

9.1 ± 3.9 days (range, 3–14 days) after the initiation of treatment. The TMP closure rate was significantly greater in the EGF group than in the observation group (96.2% *vs.* 61.1%, respectively, P = 0.026). The mean closure time was significantly shorter in the EGF group than in the observation group (P < 0.01). Purulent otorrhea developed after treatment in one patient in the EGF group but resolved with oral amoxicillin and ofloxacin eardrops. In addition, the mean levels of hearing improvement after 6 months were 12.1 ± 6.2 dB for the EGF group and 11.7 ± 4.8 dB for the observation group for the patients with TMP closure. The difference in hearing improvement rate between the two groups was not statistically significant (*t*-test, P = 0.86).

4. Discussion

Most traumatic TMPs heal spontaneously within 8 weeks, with a significantly lower subsequent healing rate [1,11,12]. Griffin [12] reported that almost 70% of traumatic TMPs healed spontaneously within 30 days, and that this healing rate decreased to 18% in the subsequent months. Orji [1] reported that 81% of perforations closed within 2 months, and the closure rate decreased to 13% during the subsequent 2 months. In our previous study, 83.3% of perforations closed within 2 months, and the closure rate decreased to 21.1% during the subsequent several months [11]. A TMP that fails to heal within 4 weeks after trauma has been defined by some authors as a traumatic subacute TMP [10].

Growth factors have been widely used to repair TMPs in recent years [4–10,13–15]. The topical application of EGF was reported to accelerate the closure of human and animal acute TMPs [4–9]. Nevertheless, the effects of EGF on chronic TMPs remain controversy. EGF accelerates the closure of animals chronic TMPs [13–15] while it didn't improve the closure of human chronic TMPs [16]. Although Ramalho and Bento [10] reported that topical application of EGF facilitated the healing of animal subacute TMPs, the efficacy of EGF in human subacute TMPs is also unclear. We used EGF to repair human subacute TMPs that did not close completely within 8 weeks in this study. Although most of subacute TMPs showed closure of some degree during the first two months, these perforations didn't achieve to completely close, some perforations showed the excessive proliferation of epithelium at the perforation edge. Previous studies suggested that the outward migration and excessive proliferation of epithelium at the perforation edge resulted in failed or delayed healing of human subacute TMPs [17,18]. The results showed that the topical application of FGF significantly improved the closure rate and shortened the closure time compared to observation only. The present study indicated that topical application of EGF was effective to facilitate the healing of human subacute TMPs. It is well known that hyperkeratotic epidermal tissue resulted in failed or delayed healing because of the absence of blood vessels and a fibrous layer. The topical application of EGF facilitated the

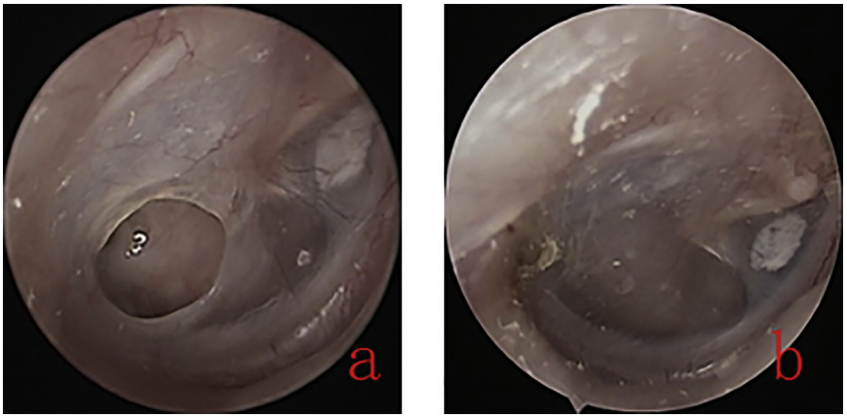

Fig. 2. The healing of medium-sized perforations after EGF treatment: 3 months after perforation (a), and 1 week after treatment (b).

recovery of a normal TMP healing process, as well as the proliferation and migration of epithelial cells and fibroblasts and revascularization, thereby accelerating eardrum healing [4]. In addition, topical application of EGF is simple, time saving, inexpensive, and suitable as an outpatient procedure. In China, one bottle of EGF drops costs ¥23.0 CNY (approximately US$4.0), and each patient requires only one bottle of EGF drops for treatment. Therefore, EGF treatment is less expensive than myringoplasty (tympanoplasty), and the application of EGF is worthwhile from a practical perspective. However, this study was limited by the small sample size because of very few cases with subacute perforation in otology clinic.

5. Conclusion

Our results suggest the feasibility of the direct application of EGF to human subacute TMPs, this method significantly improved the closure rate and shortened the closure time of subacute TMPs. In addition, the margin of the perforation does not need to be trimmed prior to EGF treatment. Thus, the technique is simple, convenient, and can be performed on an outpatient basis, avoiding the need for myringoplasty in some patients.

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Conflict of interest

No.

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