Acta Otolaryngol (Stockh) 1996; 116: 589–593

Lysates from Cultured Allogeneic Keratinocytes Stimulate Wound Healing after Tympanoplasty*

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Somers T, Verbeken G, Vanhalle S, Delaey B, Duinslaeger L, Govaerts P, Offeciers E. *Lysates from cultured allogeneic keratinocytes stimulate wound healing after tympanoplasty.* Acta Otolaryngol (Stockh) 1996; 116: 589–593.

In the past, cultured keratinocyte allografts have been used with benefit in the treatment of burn wounds and leg ulcers. Since in burn wounds autologous and allogeneic fresh keratinocyte cultures were found to give similar favorable results as lysates of allogeneic cultured cells, the authors investigated whether this lysate mixed in an antibiotic suspension would also accelerate the epithelial healing after routine tympanoplasty. In a double blind setting the healing process in 50 consecutive tympanoplasty ears was studied: an acceleration of healing of 8 days was observed in the lysate-treated group (39.25 days) as compared with the control group (47.23 days). The percentage of ears which healed within 6 weeks (after 5 weekly applications of 200 μl suspension in both groups) was significantly higher in the treated group (61%) than in the control population (36%). Although the therapeutical effect of the keratinocyte lysate in this study is believed to be due primarily to its mitogenic activity through growth factors or cytokines, at present it is still unclear which growth factors are involved and which combinations of these factors have to be present to modulate the different stages of the complex healing processes. *Key words*: *tympanoplasty, middle ear surgery, tympanic membrane, wound healing, keratinocyte, growth factor, cytokine.*

INTRODUCTION

The goal of all tympanoplasty procedures is first to eradicate the pathology and secondly to restore a strong but vibrating barrier between the external ear with its epithelial lining and the middle ear with its mucosal lining. Further hearing improvement is achieved by ossiculoplasty using biological or biocompatible materials.

The success of a tympanoplasty procedure is, amongst many factors, largely depending on the speed and quality of the healing mechanisms. The wound healing on a tympanic graft is accomplished by migration of epithelial cells along the graft and invasion of the graft by mesenchymal cells and capillaries (1). The epithelium in the depth of the outer ear canal and annular region presents an unusually proliferative nature as demonstrated by the use of monoclonal antibodies specific for cytokeratin 16, which is a hyperproliferative marker (2). So, at regular otomicroscopical controls after tympanoplasty, a centripetal growth of new epithelium is routinely observed. The tympanic graft acts as a scaffold and must show sufficient structural strength to whitstand the tendency to necrosis until the different new layers of the drum have been restored. The faster the graft is vitalised, the lesser the chance for reperforation.

We searched for a way to stimulate this epithelial healing process after tympanoplasty.

The use of cultured epidermal cell sheets is a recognized method for the coverage of extensive burn wounds and chronic leg ulcers (3–5). In burn wounds, Duinslaeger et al. (3) showed that the epithelialisation of the interstices of meshed skin autografts superimposed with sheets of autologous or allogeneic cultured keratinocytes is accelerated two-fold as compared with wound beds covered only with meshed autografts. This two-fold stimulation of epithelialisation is documented not only with autologous and allogeneic fresh keratinocyte cultures, but also with lyophilised allogeneic keratinocyte lysates. It is speculated that the keratinocytes contain growth factors or cytokines which are able to promote migration and proliferation of resident cells at the wound edges. In a previous study (6) we used fresh cultured keratinocyte sheets to treat therapy-resistent chronic postoperative otorrhoea and obtained epithelial healing in 69% of the cases.

The present paper reports on a double blind study where the healing process after tympanoplasty in ears instilled with a routinely used antibiotic ototopical medication (TerraCortril® Pfizer) was compared with the epithelial healing in ears instilled with the same eardrops to which lyophilized allogeneic keratinocyte lysate was added.

MATERIAL AND METHODS

Patient selection, randomization and statistics

Between June and November 1994, a total of 57 consecutive tympanoplasty cases using the closed

* Partially presented at the Centennial Meeting of the Belgian ENT-society, Charleroi, 17 June 1995.

 ISSN 0001-6489

For preparation of the placebo control syringes, 26 ml of pyrogen-free phosphate-buffered saline was mixed with 44 ml of TerraCortril suspension and dispensed in syringes as described above.

RESULTS

Of the final 50 ears, 28 received the lysate and 22 the placebo.

The ears instilled with TerraCortril containing lyophilised keratinocyte lysate healed 8 days faster (39.25 days) than the ears were only TerraCortril was used in the control group (47.23 days) (Table I). The difference was found to be statistically significant (ANOVA: p-value = 0.0271).

The minimal and maximal duration of healing varied in both groups in the same order of magnitude while the standard deviation (S.D.) and the standard error of the mean (S.E.M.) were very close in both groups (S.D.: 12.68 and 11.96; S.E.M.: 2.7 and 2.26 in control and lysate groups, respectively).

Fig. 2 shows that the percentage of ears which healed within 6 weeks was greater (61%) in the lysate treated group than in the control group. After 4 and 5 weeks the numbers were almost double in the lysate treated group compared with the control group (after 5 weeks: 50% and 27%, after 4 weeks: 18% and 9%, respectively). Statistical parameters such as the age of the patient, and the indication for surgery did not play a significant role. Of the initial 57 cases, 4 were rejected for statistical analysis because of reperforation, making the determination of the exact time of the epithelial closure impossible. Three of those cases were in the control group, one in the lysate treated group.

Table I. *The results in 50 tympanoplasties show a 8 days' faster healing in the lysate group than in the controls*

	Mean	SD	SEM	Min	Max
Lysate group	39.25 days	11.96	2.26	21	63
Control group	47.23 days	12.68	2.70	26	66

Anova: p-value = 0.0271.

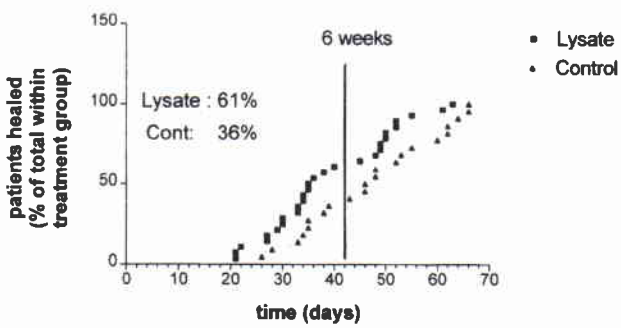

Fig. 2. The results presented according to the number of ears which healed within a defined period of time (e.g. after 6 weeks: 61% were healed in the treated group, 36% in the control group).

DISCUSSION

Up to now many otological studies have focused on the healing process of the drum membrane and the effect of the graft on this process (1). Since more and more data is becoming available concerning the complex wound repair mechanisms in general there is a growing interest in how this process could be accelerated in order to treat chronic wounds or to improve healing after surgery. Proops et al. used tissue culture as a research tool to study the migratory characteristic of the skin of the ear canal. In this study we used tissue culture as a means of improving healing after tympanoplasty. The ear operated on by the closed technique and with the use of tympanic homografts, as advocated by our teacher Jean Marquet (7), is the ideal model to study the epithelial healing (Fig. 1). After total myringectomy the surface area to be covered by the epithelium is rather constant while this is not the case after fascia tympanoplasty or radical mastoid surgery where the uncovered surface area shows greater variability.

In 1975, Rheinwald & Green described the method allowing the successful serial subcultivation of human keratinocytes (8). Since then, *autologous cultured keratinocyte grafts* have been used for the treatment of large burns (3, 5, 10, 11), skin ulcers and junctional epidermolysis bullosa (11). The great advantage of the technique is the provision of a virtually unlimited quantity of graftable autologous epithelium for permanent wound coverage in patients presenting large wounds for whom there are few other therapeutic options.

In otology, autologous cultured keratinocyte grafts have been used by Premachandra et al. (12) to cover granulating and discharging mastoid cavities with a 65% success rate. The main disadvantages of these grafts are: the need for a donor site on the patient to be treated, the delay (2 to 3 weeks) for cell culture, and the fact that repeated grafting, if need be, causes discomfort to the patient because a skin biopsy has to be taken for each culture.

A more recent development in the field of tissue culture is the use of cultured epithelium derived from an allogeneic donor (3, 4, 5, 10, 11). This technique of *allogeneic cultured keratinocyte grafting* has been found beneficial with similar efficiency as autologous

4. Marcusson J, Lindgren C, Berghard A, Toftgärd R. Allogeneic cultured keratinocytes in the treatment of leg ulcers: a pilot study. Acta Derm Venereol (Stockh) 1992, 72: 61–4.
5. De Luca M, Albanese E, Bondanza S et al. Multicentre experience in the treatment of burns with autologous and allogenic cultured epithelium, fresh or preserved in a frozen state. Burns 1989; 15–5: 303–9.
6. Somers T, Verbeken G, Vanhalle S et al. Treatment of chronic postoperative otorrhea with cultured keratinocyte sheets. Ann Otol Rhinol Laryngol (in press).
7. Marquet J. Twelve years' experience with homograft tympanoplasty. Otolaryngol Clin North Am 1977: 10: 581–93.
8. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of kertinizing colonies from single cells. Cell 1975; 6: 331–44.
9. Proops DW, Hawke WM, Parkinson EK. Tissue culture of migratory skin of the external ear and cholesteatoma: a new research tool. Otolaryngology 1984; 13: 63–9.
10. Cuono C, Langdon R, Mc Guire J. Use of cultured autografts and dermal allografts as skin replacement after burn injury. Lancet 1986; *i*: 1123–4.
11. Phillips T. Cultured skin grafts. Arch Dermatol 1988; 124: 1035–8.
12. Premachandra DJ, Woodward B, Milton CM, Sergeant RJ, Fabre JW. Long-term results of mastoid cavities grafted with cultured epithelium prepared from autologus epidermal cells to prevent chronic otorrhea. Laryngoscope 1993; 103: 1121–5.
13. Morhena VB, Benike CJ, Cox AJ. Cultured human epidermal cells do not synthetize HLA. J Invest Dermatol 1982; 78: 32–7.
14. Burt AM, Pallett CD, Sloane JP et al. Survival of cultured allografts in patients with burns assessed with probe specific for Y chromosome. BMJ 1989; 298: 915–9.
15. Eisinger M, Sadan S, Silver IA, Flick RB. Growth regulation of skin cells by epidermal cell-derived factors: implications for wound healing. Proc Natl Acad Sci USA 1988; 85: 1937–41.
16. Kratz G, Haegerstrand A, Dalsgaard C. Conditioned medium from cultured human keratinocytes has growth stimulatory properties on different human cell types. J Invest Dermatol 1991; 97: 1039–43.
17. Spandow O, Hellström S. Animal model for persistent tympanic membrane perforations. Ann Otol Rhinol Laryngol 1993; 102: 467–72.
18. Lee A, Jackler R, Kato M and Scott N. Repair of chronic tympanic membrane perforations using epidermal growth factor: progress toward clinical application. Am J Otol 1994; 15: 10–8.
19. Vrabec J, Schwaber M, Davidson J, Clymer M. Evaluation of basic fibroblast growth factor in tympanic membrane repair. Laryngoscope 1994; 104: 1059–64.
20. Mondain M, Ryan A. Histological study of the healing of traumatic tympanic membrane perforation after basic fibroblast growth factor application. Laryngoscope 1993; 103: 312–8.

Submitted September 21, 1995; accepted November 22, 1995

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