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Short note

Effect of injection of triamcinolone on type 1 procollagen and fibronectin in scar tissue

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Hypertrophic and keloid scar formation are common following median sternotomy. The authors have previously identified a biochemical marker in scars, namely type 1 procollagen peptide, which correlates with the macroscopic appearance of sternotomy scars[1]. Antibodies to type 1 procollagen recognize the portion of the amino- or carboxyl-terminal domains of type 1 procollagen molecule which are proteolytically removed during collagen secretion. Thus, antibody staining identifies cells actively synthesizing type 1 collagen and hence contributing to scar formation.

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The present study sought to determine whether measurement of type 1 procollagen peptide could be used in the evaluation of methods of scar reduction. This study aimed to discover whether injection of corticosteroids into a wound 6 weeks after operation would reduce the abundance of type 1 procollagen staining.

Patients and methods

Twenty patients were recruited 6–8 weeks after undergoing median sternotomy for cardiac surgery. The standard surgical technique employed was incision of skin by scalpel and of deep layers by diathermy with skin closure using subcuticular 2/0 polyglycolic acid sutures. Patients were given flucloxacillin for 5 days and either gentamicin or cefuroxime for 48 h over the operative period according to renal function. Two randomly allocated 2·5 cm regions in the lower 10 cm of the scar were injected with 0·1 ml drug solution or 0·1 ml saline. Drug solutions were either triamcinolone acetonide (Lederle Laboratories, Gosport, UK) 10 mg/ml (11 patients) or 5 mg/ml (nine patients).

One week after injection a 3-mm punch biopsy was taken from both drug and placebo injection sites. Immunochemical examination was performed with standard methods[1] on formalin-fixed paraffin sections of the biopsies using antibodies against type 1 procollagen (Chemicon International, Temecula, California, USA), transforming growth factor (TGF) β (R & D Systems, Abingdon, UK), fibronectin (Sigma Chemicals, Poole, UK) and α smooth muscle actin (Dako, High Wycombe, UK). All the secondary antibodies and the ABC kit were obtained

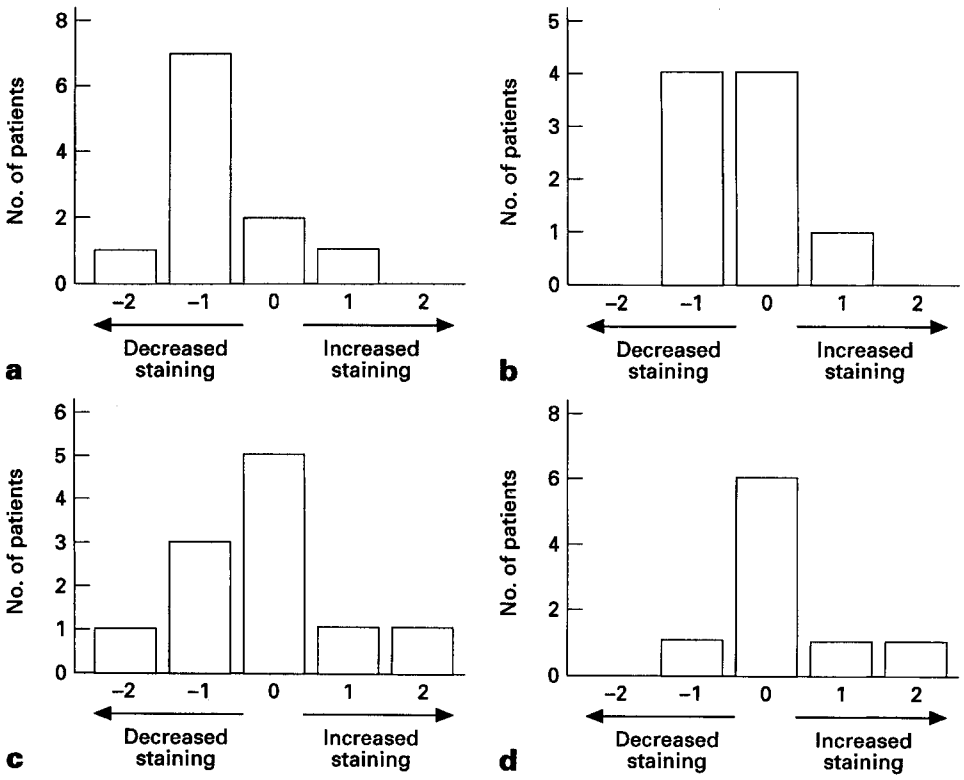

Fig. 1 Number of patients demonstrating a change in the grade of type 1 procollagen immunostaining after **a** high-dose and **b** low-dose triamcinolone, and in the grade of fibronectin staining after **c** high-dose and **d** low-dose triamcinolone, compared with saline. A high dose (**a** and **c**) consisted of a 0·1 ml injection of a 10 mg/ml triamcinolone solution and a low dose (**b** and **d**) 0·1 ml of a 5 mg/ml solution

from Dako. Grading of sections was performed by a pathologist blind to the code, using a scale of 0, +/−, +, + +, + + +. Biopsies from drug- and placebo-treated regions of the same patient were compared and the difference in the staining grade recorded. *In situ* hybridization for procollagen messenger RNA (mRNA) was performed as described previously[2].

The study was approved by the local ethics committee. Statistical analysis was carried out using the Wilcoxon matched-pairs signed rank test.

Results

By means of haematoxylin and eosin staining, scars were seen to comprise focal aggregates of chronic inflammatory cells in scar tissue. Immunohistochemical staining for type 1 procollagen identified collagen-producing fibroblasts in both cellular matrix and perivascular areas. *In situ* hybridization confirmed the presence of type 1 collagen mRNA in these sections from post-sternotomy scars. There was binding of the probe to cells within the matrix, which was absent when sections were treated with ribonuclease before hybridization.

Local injection of the higher dose (10 mg/ml) of triamcinolone resulted in a significant reduction in immunostaining for type 1 procollagen compared with placebo (*Fig. 1*). This reduction was not observed with the lower dose of triamcinolone (5 mg/ml). There was little change in fibronectin staining following administration of triamcinolone. Staining using antibodies against TGF-β and α smooth muscle actin was weak in all sections and did not alter with therapy. No macroscopic differences in the area of scar treated by either triamcinolone or placebo were observed.

Discussion

The authors' previous study[1] suggested that measurement of type 1 procollagen in a wound might serve as a biochemical marker for the intensity of the scarring process and that this might correlate with macroscopic appearance. In the present study, local injection of a relatively high dose of corticosteroid altered the intensity of type 1 procollagen staining in the scar and, by inference, might reduce the intensity of the subsequent scarring process. The observation that corticosteroids reduce type 1 collagen production agrees with other *in vitro* studies[3], although fibroblasts from hypertrophic or keloid scars may be relatively refractory to corticosteroids[4]. Local injection of corticosteroids has been used to prevent hypertrophic scarring[5], although there is concern that this approach may impair wound healing.

The use of a biochemical marker of new collagen production in short-term studies on the intensity of the scarring process may offer a way to develop and assess new therapies to prevent the clinical problem of disfiguring scars.

Acknowledgements

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