Neutrophil migration through denuded skin areas: a non-invasive, quantitative and reproducible method to study epidermal cicatrization \textit{in vivo} in man

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Wound healing is a very complex phenomenon involving blood, dermal and epidermal factors. These factors are tightly associated since, for example, dermo-epidermal injuries do cicatrize at a lower rate than same sized pure epidermal wounds (Pollack, 1979). On the other hand, \textit{in vivo} studies of skin cicatrization are of critical interest since numerous pathological, pharmacological or physical conditions, such as occlusion by polyethylene film (Eaglstein & Mertz, 1978), do modify wound healing.

In order to undertake \textit{in vivo} studies in man for normal or pathological skin cicatrization with or without therapeutic agents, one has to use experimental procedures which fit at least three often conflicting conditions. Firstly, these procedures have to be related to the entire complex phenomenon. Secondly, the methods should give quantitative and reproducible data and, if possible, a kinetic evaluation. Thirdly, experimental procedures have to be as non-invasive as possible in order to be acceptable by human volunteers. Histological procedures have been already performed after dermo-epidermal injuries (Winter, 1972). They gave important qualitative results with regard to the entire phenomenon. However, they are invasive and therefore have been essentially performed on animals. Moreover, as far as histological procedures are concerned, quantitative evaluation is difficult. Therefore more analytical and quantitative experimental procedures are of interest.

Using suction blisters, which create pure and reproducible epidermal wounds and leave the basal lamina intact (Ortonne, 1980; Dubertret, Lebreton & Touraine, 1982a, b), one may look more selectively at the epidermal events of skin cicatrization. Such experiments may be qualitative if histological procedures are used and quantitative if planimetric evaluations are performed after dermo-epidermal separation (Eaglstein & Mertz, 1978; Fourtanie et al., 1984). However, both procedures are invasive.

The aim of our work was to develop a reproducible and non-invasive method allowing quantitative analysis of human epidermal healing. The method we propose is based on the repair of the epidermal barrier function after purely epidermal wounds (Saiag et al., in preparation).

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Reproducible purely epidermal wounds were produced by mild suction (-500 g/cm²). After removal of the blister roofs, Macrolon sterile skin chambers were stuck over the denuded areas and filled with 1 ml of sterile collecting medium. Until migration became undetectable, the migrating neutrophils (PMN) were counted every 24 h and the collecting media replaced by fresh ones, thus providing a constant composition.

In order to study the reproducibility of the measurements, two suction blisters of 0.125 cm² were performed on each forearm of 10 individuals (aged 21–25 years). Hank’s medium (HM) and autologous serum (AS) were used to fill, respectively, the first and second chamber on each forearm in order to compare the rate of cicatrization in these two media.

In another set of experiments, in order to compare the healing of different sized wound areas, suction blisters of 0.125 and 0.5 cm² were performed simultaneously on one forearm of nine other volunteers (aged 20–27 years) and M199 was used as collecting medium.

RESULTS

For all experiments, a regular decrease of neutrophil migration in the skin chambers was observed, suggesting a progressive decrease of the area available for PMN migration. Biopsies were performed when the migration had stopped. Total epidermal healing involving a normal stratum corneum and a slight hypergranulomatosis was observed.

After wounds of 0.125 cm², this epidermal healing was achieved in 5.5 ± 0.6 days (mean ± s.d.) with HM as collecting medium and in 5.8 ± 0.7 days with AS. These results were not significantly different (Student’s t-test for paired values). The coefficient of variation of the measurements between the two homologous skin chambers filled with the same medium was calculated as 11 ± 6%, showing the reproducibility of the technique.
Epidermal cicatrization in vivo in man

In the second set of experiments, where M199 was used as collecting medium, total epidermal healing was achieved in $7.6 \pm 0.5$ and $6.3 \pm 0.4$ days, respectively, after wounds of $0.5$ and $0.125$ cm$^2$. These data were significantly different (Student's $t$-test for paired values; $P < 0.01$).

For same-sized wound areas, the total cumulative number of migrating PMN depended on individuals and on the collecting medium used (PMN were much more numerous if AS was used). When data of each experiment were expressed as cumulative PMN/plateau value, the kinetics of the different curves could be compared and showed high homology whatever the subject (Fig. 1).

DISCUSSION

In these experiments, the parameter we have studied was PMN migration through denuded skin areas. This parameter is relevant to epidermal wound healing since biopsies performed when migration had stopped showed total cicatrization. Moreover, since we renewed the collecting media every 24 h, thus providing rather constant chemotactic stimuli, the observed decrease of PMN migration is relevant to the decrease of the skin area available for their migration. Therefore, our method allows the study of the repair after purely epidermal injuries of the key function of the stratum corneum, the impermeability, with respect to its kinetics. This non-invasive technique gave quantitative and reproducible results, which could be used for studying in vivo the consequences of drugs on human epidermal wound healing. Moreover, it is simple to establish during the first 24 h of each experiment whether PMN functions are modified by the drug tested (Dubertret, Lebreton & Touraine, 1982a, b). That could be important since PMN are involved in obtaining an efficient antibacterial activity at the wound level as well as an efficient cleaning of the wounded area.

However, some limitations of the technique are obvious. Firstly, only epidermal cicatrization is studied. Secondly, wound healing occurs under occlusive migration chambers filled with a liquid medium. These conditions are somewhat different from the usual ones. Therefore, attention must be paid before extrapolating the results obtained with this technique.

REFERENCES


