J. Pharm. Pharmacol. 1993, 45: 784–790
Received May 12, 1992

Skin Wound Healing: Some Biochemical Parameters in Guinea-pig

F. BUFFONI, G. BANCHELLI, S. CAMBI, G. IGNESTI, R. PIRISINO, L. RAIMONDI AND G. VANNELLI*

Department of Pharmacology, University of Florence, Viale G. B. Morgagni, 65-50134 Firenze, Italy, and
**Department of Human Anatomy, University of Florence, Italy*

Abstract—Hairs were removed from the dorsal skin of guinea-pigs and 5–6 wounds (7 × 7 mm) were surgically induced by totally removing the epidermal and part of the dermal surface. They were then allowed to heal. The newly formed wound tissues were dissected at different times during the process and analysed by biochemical and histological methods. Hydroxyproline, proteins, DNA and semicarbazide-sensitive amine oxidase (SSAO) were measured, as were [^{14}C]leucine and [^{3}H]thymidine incorporation in some samples. The peroxidase-like activity of plasma albumin and the histology of wounds stained with haematoxylin-eosin were also studied. It was shown that SSAO enzymes, which are present in normal guinea-pig skin and have a high affinity for benzylamine are localized in fibroblasts. During skin healing in the newly formed tissue there was an increase in protein content which reached a maximum after 4–6 days; DNA content also increased. The rate of incorporation of [^{3}H]thymidine and [^{14}C]leucine paralleled DNA and protein content, respectively. The content of hydroxyproline had greatly decreased with respect to that in normal skin after 2–10 days. SSAO activity increased much less than DNA after 4 days whereas after 10–11 days it increased more than DNA, thus indicating that at this time it was probably produced by fibroblasts. No significant increase in the peroxidase-like activity of albumin was observed 4, 8 or 11 days after surgery. Treatment of the animals with methylprednisolone acetate (20 mg kg^{-1}, i.m.) two days before surgery decreased the rate of skin healing but did not alter the level of albumin peroxidase activity of the plasma. Histology showed that in the animals treated with this drug the re-epithelialization was slower and after 11 days the wound appeared similar in appearance to the 8-day control wounds. In the methylprednisolone-treated animals a positive correlation was observed between the DNA content of regenerating tissue and the hydroxyproline content, whereas a negative correlation was observed in the control wounds. This correlation was in full agreement with the histological observation which showed an increased amount of cytogen collagen in the wounds of the treated animals. The simultaneous study of the biochemical parameters (DNA, proteins, SSAO, hydroxyproline) appears to be a good method for differentiating the pharmacological effects on the different cells which are responsible for wound healing.

Wound healing is restoration of the continuity of living tissue. It is a product of the integrated response of several cell types to injury. It involves platelet aggregation and blood clotting, formation of fibrin, an inflammatory response to injury, alteration in the ground substance, endothelial and capillary proliferation and surface covering, regeneration of certain cell types, variable contracture and remodelling. Healing is not complete until the disrupted surfaces are firmly knit by collagen.

A variety of wound models has been employed to study this process (Schilling 1968; Bouchlier et al 1990). The main purpose of this work was that of studying a procedure suitable for the in-vivo quantitative evaluation of pharmacological effects on the rate of skin regeneration and wound healing.

In addition to the classical methods which detect the collagen content, the number of cells in the new connective tissue and epithelial layers, this paper introduces the measurement of some biochemical parameters and two new procedures: the determination of semicarbazide-sensitive amine oxidase (SSAO) activity and the determination of peroxidase-like activity of the plasma albumin.

Correspondence: F. Buffoni, Department of Pharmacology, University of Florence, Viale G. B. Morgagni, 65-50134 Firenze, Italy.

The SSAOs (E.C.1.4.3.6) comprise a large class of enzymes including diamine oxidase, plasma benzylamine oxidase and spermine oxidase and many tissue bound enzymes which generally have a very high affinity for benzylamine. As we have recently shown, they differ in the pattern of substrate specificity in the various tissues and in different species (Ignesti et al 1992). The physiological significance of these enzymes is still under investigation but certainly these enzymes seem to increase during capillary formation (Ziche et al 1987). This study might therefore contribute to a better understanding of the role of SSAO and eventually to the introduction of a new useful parameter describing the skin healing process. The measurement of the peroxidase-like activity of albumin which has been described by Pirisino et al (1988) might be a marker of the degree of inflammation.

Materials and Methods

Materials

Male guinea-pigs (300 g, Dunkin-Hartley) were obtained from Rodentia (Torre Pallavicina, Italy).

[7-^{14}C]Benzylamine hydrochloride (54 mCi mmol^{-1}) was purchased from Amersham (Buckinghamshire, UK); [^{14}C(U)]leucine (325 mCi mmol^{-1}) and [6-^{3}H]thymidine (15 Ci mmol^{-1}) were purchased from Du Pont de Nemours (Dreiech, Germany).

Dulbecco's modified Eagle medium (MEM) and foetal calf serum were obtained from Gibco (Mascia Brunelli Spa., Milano, Italy).

Deoxyribonucleic acid (DNA) was purchased from Boehringer Mannheim (Penzberg, Germany). Pargyline hydrochloride, hydroxyproline, calf serum albumin (fraction V), guinea-pig serum albumin, *tert*-butylhydroperoxide and anti-rabbit immunoglobulin-peroxidase conjugate were purchased from Sigma (St Louis, MO, USA). Chloramine T, *p*-dimethylaminobenzaldehyde and 2,2′-dinitro-5,5′-dithiodibenzoic acid (DTNB) were purchased from Merck (Frankfurt, Germany). Bisbenzimide (H 33258) was purchased from Fluka (Buchs, Switzerland). 2-Nitro-5-mercaptobenzoic acid (NMBA) was prepared according to Ignesti & Coppi (1981). Antibodies against pure pig plasma benzylamine oxidase were prepared according to Buffoni et al (1977). The kit for albumin determination was purchased from Abbott Laboratories (North Chicago, IL, USA). Methylprednisolone acetate (Depo-Medrol) was from Upjohn (40 mg mL^{-1}), ketamine hydrochloride (Ketalar) was from Parke Davis (50 mg mL^{-1} base). All other reagents were of analytical grade.

Surgery

Animals were anaesthetized with ketamine (150 mg g^{-1}, i.m.). Hair was removed from the dorsal skin and the skin washed with soap and cleaned with alcohol before surgery. Five to six wounds (7 × 7 mm and of 1–2 mm thickness) were surgically induced by totally removing the epidermal and part of the dermal surface. They were covered with dressing and allowed to heal. The newly-formed wound tissues were dissected at different times during the process and analysed by biochemical and histological methods. The removed pieces of skin were quickly weighed and homogenized in a glass-glass homogenizer in ice. Each piece was homogenized in 1·5 mL water except the pieces used for SSAO determination which were homogenized in 1·5 mL 10 mM phosphate buffer, pH 7·4, containing 0·25 M sucrose, and the pieces used for ^{14}C and ^{3}H incorporation, which were homogenized in 1·5 mL MEM.

Blood was obtained by intracardial puncture with a syringe containing heparin.

Analyses

Proteins were determined according to the method of Lowry et al (1951) using bovine plasma albumin as standard.

Albumin in the guinea-pig plasma was determined using the Abbott kit.

The DNA content was determined according to the method described by Labarca & Paigen (1980).

Hydroxyproline determination was carried out according to Woessner (1961): crude tissue homogenates (0·1 mL) were diluted to 1 mL with hydrochloric acid to a final concentration of 6 M.

The samples were sealed in Pyrex hydrolysis tubes and hydrolysed for 3 h at 130°C. Tubes were then opened and the contents were decanted into a graduated tube, brought to pH 6–7 and to the final volume of 5–10 mL; 2 mL were used for the spectrophotometric determination of hydroxyproline.

The incorporation of thymidine and leucine was carried out according to the method described by Lynch et al (1987) using 0·2 mL homogenates. A program for the simultaneous determination of ^{14}C and ^{3}H was used in a Packard scintillation counter.

The peroxidase-like activity of albumin was assayed according to Pirisino et al (1988) using 50 μL plasma and 800 μL 0·1 M phosphate buffer, pH 7·4. The decrease of the absorbance at 412 nm was measured by adding, after 10 min incubation at 37°C, 100 μL 3 mM *tert*-butylhydroperoxide.

For SSAO activity determination, crude skin homogenates were centrifuged at 600 *g* for 5 min to remove unbroken cells, nuclei and debris and then at 12000 *g* for 20 min. Supernatant (0·2 mL) was incubated for 30 min with 0·05 mL 6 mM pargyline at 37°C in order to inhibit completely the monoamine oxidase activity (A and B) of the sample, then 0·05 mL 500 μM [^{14}C]benzylamine was added. The reaction was stopped after 10 min by the addition of 0·1 mL 3 M hydrochloric acid and the aldehyde formed was extracted with 1 mL ethylacetate. Radioactivity was measured using 0·5 mL of the ethylacetate extract in 10 mL of Instagel in a Packard scintillation counter.

Histology

Samples were fixed in Bouin's solution for 24 h, then washed with 70° ethyl alcohol. The fixed samples were embedded in paraffin and sections made perpendicular to the skin surface and stained with haematoxylin and alcoholic eosin. The immunoperoxidase method was used for localization of tissue SSAO using rabbit antibodies against pig plasma benzylamine oxidase, which cross-react with the tissue SSAO.

Statistical analysis

Kinetic constants of SSAO were obtained by the method of Wilkinson (1961). Results were analysed statistically using the computer program published by Tallarida & Murray (1981).

Results

SSAO activity of guinea-pig dorsal skin

In 46 samples of guinea-pig normal dorsal skin the SSAO activity was $15 \pm 0{\cdot}9$ pmol (mg protein)$^{-1}$ min^{-1} (mean ± s.e.). This enzymic activity showed a high affinity for benzylamine (K_m $5{\cdot}3 \pm 1{\cdot}4$ μM, V_{max} $15 \pm 1{\cdot}0$ pmol (mg protein)$^{-1}$ min^{-1} (mean ± s.e. of three experiments, each on four pieces of skin)).

Histochemical identification of the cells containing SSAO

As shown in Fig. 1, the antibodies against pig plasma benzylamine oxidase reacted with a protein which was present in the stromal cells of guinea-pig dorsal skin. These cells (fibroblasts) had a stellar configuration and were mainly localized in the dermal layer, but some were also present between epidermal cells.

Changes in some biochemical parameters during wound healing

The process of skin regeneration in the dorsal skin of guinea-pig was characterized by an early increase in protein, DNA, SSAO activity and by a parallel increase of the [^{14}C]leucine and [^{3}H]thymidine incorporation, which reached a maxi-

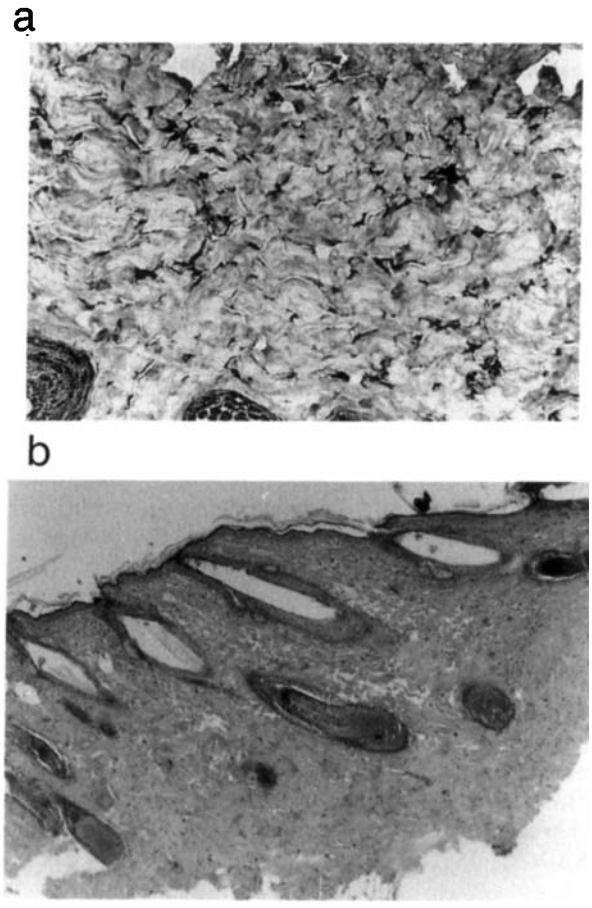

FIG. 1. Immunohistochemical identification of the cells containing SSAO (a) and histology of the normal skin (haematoxylin-eosin) (b). In a the dark staining is localized in the cells of the derma.

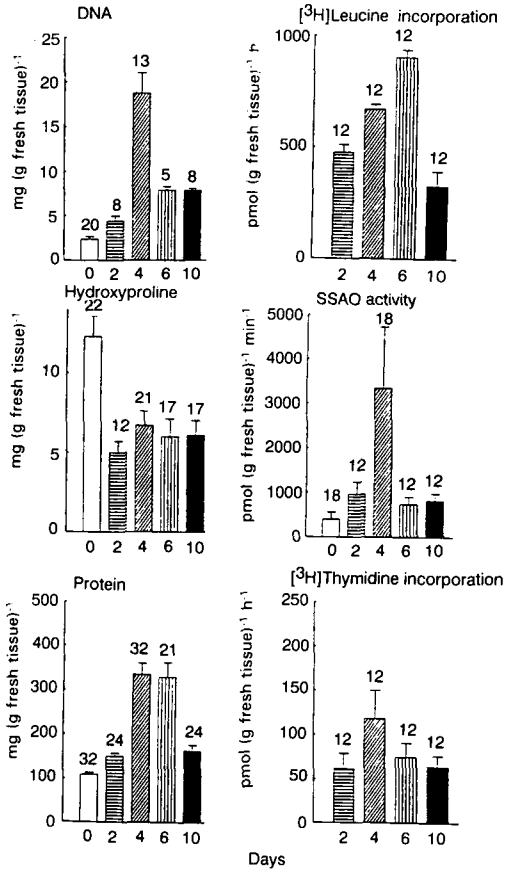

FIG. 2. Some biochemical parameters during regeneration of the dorsal skin of guinea-pig (mean ± s.e.). 0 = skin removed during the first surgery. The number on each column indicates the number of samples. All values were significantly different from the corresponding zero time sample ($P < 0.05$).

mum 4 days after surgery (Fig. 2), whereas the hydroxyproline content was decreased at each time studied. Ten days after surgery, proteins and DNA were still above control values, whereas the incorporation of [^{14}C]leucine in proteins and of [^{3}H]thymidine in DNA was already strongly decreased, thus indicating a lower rate of cell proliferation.

During the first 4 days after surgery there was a positive correlation between DNA content and protein content, and DNA content and SSAO activity, whereas the correlation was negative between DNA and hydroxyproline. These observations are in full agreement with the histological

Table 1. DNA content and SSAO activity of guinea-pig dorsal skin at different times after surgery in four different series of experiments (mean ± s.e., in parentheses the numbers of samples of skin, 2 per animal).

	Days		
	0	4	11
DNA (mg (g tissue)$^{-1}$)	2·7 ± 0·4 (15)	17·1 ± 1·9 (7)*	—
SSAO (pmol (g tissue)$^{-1}$ min^{-1})	380·0 ± 36 (20)	716·0 ± 152 (8)*	—
SSAO (pmol (mg protein)$^{-1}$ min^{-1})	18·8 ± 4·3 (20)	4·5 ± 1·1 (8)*	—
DNA (mg (g tissue)$^{-1}$)	1·9 ± 0·1 (30)	13·0 ± 1·1 (10)*	1·8 ± 0·2 (10)
SSAO (pmol (g tissue)$^{-1}$ min^{-1})	498·0 ± 25 (30)	642·0 ± 58 (10)	1020·0 ± 106 (10)*
SSAO (pmol (mg protein)$^{-1}$ min^{-1})	24·9 ± 1·3 (30)	5·6 ± 0·9 (10)*	32·3 ± 3·6 (10)*
DNA (mg (g tissue)$^{-1}$)	2·2 ± 0·2 (30)	—	3·5 ± 0·2 (10)*
SSAO (pmol (g tissue)$^{-1}$ min^{-1})	513·0 ± 50 (30)	—	1302·0 ± 216 (10)*
SSAO (pmol (mg protein)$^{-1}$ min^{-1})	25·4 ± 2·8 (30)	—	54·8 ± 7·9 (10)*
DNA (mg (g tissue)$^{-1}$)	2·3 ± 0·2 (30)	14·7 ± 1·3 (10)*	3·0 ± 0·2 (10)
SSAO (pmol (g tissue)$^{-1}$ min^{-1})	559·0 ± 66 (30)	783·0 ± 153 (10)	1128·0 ± 185 (10)*
SSAO (pmol (mg protein)$^{-1}$ min^{-1})	27·2 ± 3·6 (30)	10·3 ± 1·8 (10)*	64·7 ± 16·2 (10)*

* $P < 0.05$ compared with value at time zero.

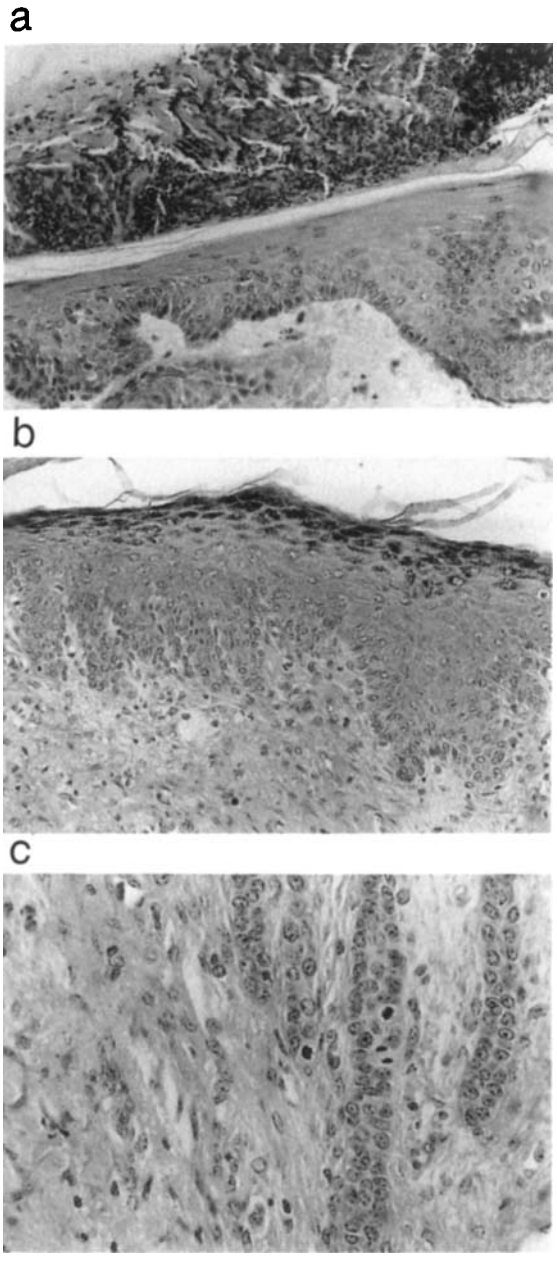

FIG. 3. Histology of the dorsal skin of guinea-pig: (a) 4 days after surgery, haematoxylin-eosin, 20×; (b) 8 days after surgery, haematoxylin-eosin 20×; and (c) 8 days after surgery, haematoxylin-eosin, 40×.

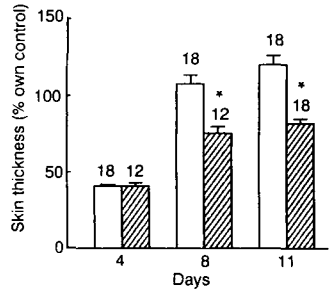

FIG. 4. Effect of methylprednisolone acetate (hatched column) on the thickness of the dorsal skin of guinea-pig at different times after surgery. Each value is expressed as % of its corresponding value at time 0; the reported values are the mean ± s.e. 0 = skin removed during the first surgery. The number on each column is the number of samples. * Difference between this value and the corresponding value obtained in the control (open column) group is statistically significant ($P < 0.05$).

results (Fig. 3). Four days after surgery an epidermal layer had already formed under the scab; at 8 days the epidermal surface was bigger and 11 days after surgery the dermal layer was more organized, although not yet normal.

The observed increase in the DNA content, which was parallel to an increase in the [^{3}H]thymidine incorporation, indicates a process of cell proliferation. Some of these cells contained SSAO activity, but these cells represented only a small part of the cells involved in the process because the increase in the DNA content and in protein was much bigger than the increase in SSAO activity which decreased if expressed per milligram of protein (Table 1) instead of per gram of fresh tissue. On the contrary, 10–11 days after surgery the SSAO activity increased more than DNA and protein and the increase was observed when the enzymic activity was expressed either as per gram of fresh tissue or per milligram of protein, thus indicating that at this time, when fibroblasts start to produce the extracellular matrix as shown by the histology, they also express SSAO activity.

Effect on wound healing of treatment with methylprednisolone

Treatment of the animals with methylprednisolone (20 mg kg^{-1}, i.m.) 2 days before surgery did not significantly change the DNA content, protein content, hydroxyproline content or SSAO enzymic activity of normal skin (Table 2). This treatment seemed able to alter the thickness of the skin (Fig. 4) during the regeneration process.

Table 2. Effect of methylprednisolone on the dorsal skin of guinea-pig, removed with the first surgical operation. Values are per g fresh tissue (mean ± s.e.).

	n	Control	n	Treated
Protein (mg)	32	109·6 ± 4·7	49	110·6 ± 3·2
DNA (mg)	20	2·4 ± 0·3	41	2·0 ± 0·2
Hydroxyproline (mg)	22	12·3 ± 1·3	40	13·5 ± 0·6
SSAO (pmol min^{-1})	18	399 ± 37	74	458 ± 20

The differences between the means are not statistically significant ($P > 0.05$). n = number of samples.

Treatment with methylprednisolone reduced the increase in DNA 8 days after surgery and increased the DNA content 11 days after surgery (Fig. 5). Four days after surgery the hydroxyproline content also increased due to methylprednisolone treatment, whereas no significant variations were observed in SSAO activity (Fig. 5).

There was a positive correlation between DNA content and hydroxyproline and this correlation clearly differentiated the regenerating process of the treated animals in comparison with controls (Table 3).

This positive correlation indicated that in the treated

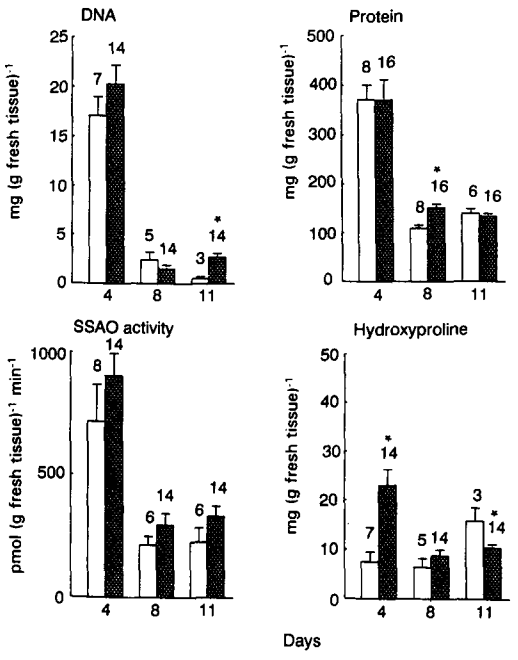

FIG. 5. Effect of methylprednisolone on some biochemical parameters of the dorsal skin of guinea-pig at different times after surgery (mean ± s.e.). * Difference between this mean and the mean obtained in the controls is statistically significant ($P < 0.05$). The number on each column is the number of samples. Control, open columns; treated, shaded columns.

Table 3. Correlation between the DNA content of the skin and other biochemical parameters in controls and in the animals treated with methylprednisolone (data from Fig. 5).

	Protein (mg g^{-1})	Hydroxyproline (mg g^{-1})	SSAO (pmol g^{-1} min^{-1})
Control	r 0·936*	r −0·540	r 0·922*
Treated	r 0·980*	r 0·936*	r 0·834*

* $P < 0.05$.

animals the process of skin healing was carried out by cells which produced the extracellular matrix. This was confirmed by histology which showed that epidermal proliferation decreased in the treated animals whereas there was an increase in cytogen collagen (Fig. 6).

Peroxidase-like activity of albumin

No significant variations in the plasma albumin content were observed in the operated animals and no variations were seen in animals treated with methylprednisolone (Table 4).

Discussion

Tissue repair is accompanied by an ordered and definable sequence of events starting with wound closure and progressing to repair and remodelling of the damaged tissue. The first

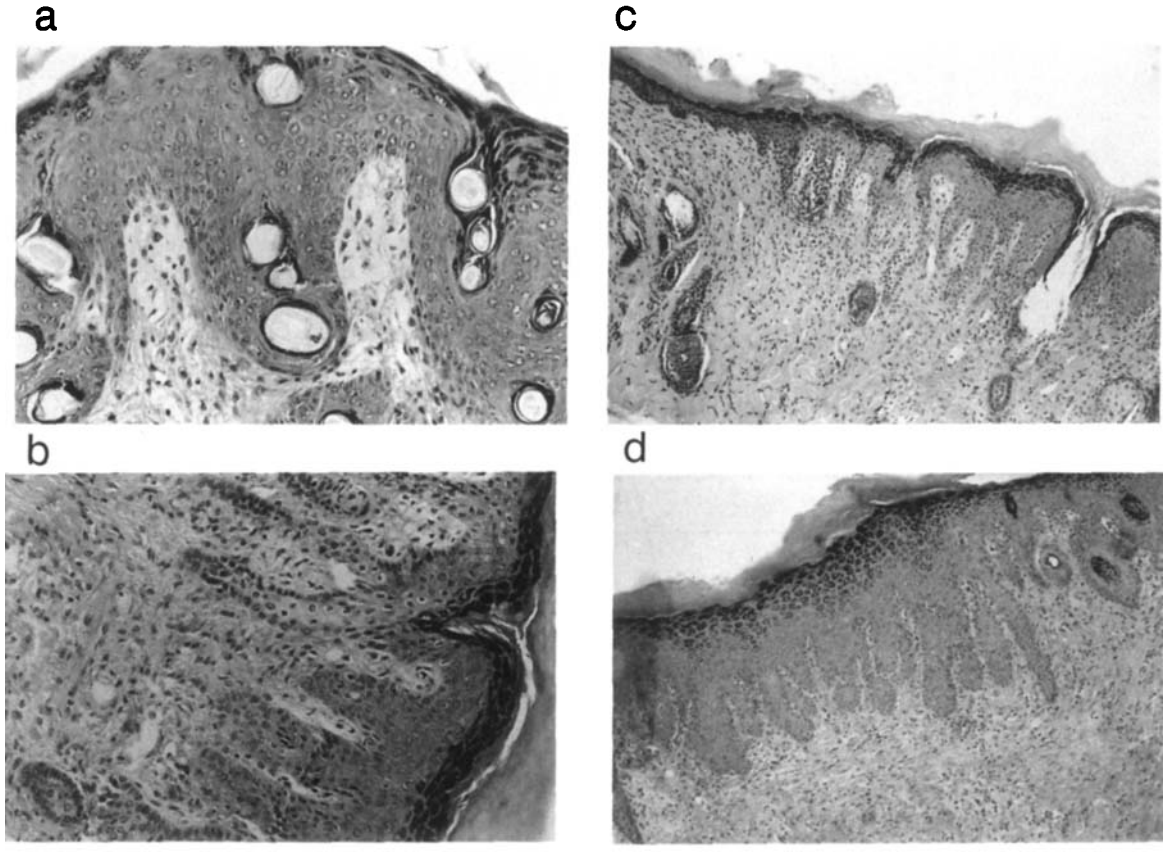

FIG. 6. Histology of the dorsal skin of guinea-pig treated with methylprednisolone (a) 4 days after surgery, haematoxylin-eosin, 20×; (b) 8 days after surgery, haematoxylin-eosin, 10×; (c) 8 days after surgery, haematoxylin-eosin, 20×; and (d) 11 days after surgery, haematoxylin-eosin, 10×.

Table 4. Effect of surgery and of treatment with methylprednisolone on the peroxidase-like activity of albumin in guinea-pig (nmol (mg albumin)$^{-1}$ min^{-1}).

Days after surgery or treatment	Surgery alone	Drug	Both
0	34·5±2·4 (14)	34·1±2·9 (8)	—
4	38·9±1·4 (15)	—	39·1±2·2 (9)
6	—	39·1±2·2 (9)	—
8	31·2±4·1 (5)	—	—
11	35·7±4·2 (4)	—	—
13	—	40·1±3·3 (8)	—

important event to take place following injury is haemostasis with platelet and clotting factor release. Platelet degranulation leads to a release of platelet-derived growth factor (PDGF) which, along with other growth factors, increases cellular chemotaxis of polymorphonuclear leucocytes, other leucocytes, macrophages and lymphocytes. Macrophages produce factors that stimulate lymphocyte proliferation and fibroblast growth and function. Finally, fibroblasts secrete collagen and fibronectin to produce the healing framework along which epithelial cells will spread to complete the wound closure.

In the guinea-pig dorsal skin there was an early increase of the DNA content of healing wounds, which appears to be not only an expression of cellular infiltration but also of cell replication because it is closely related to [H^3]thymidine incorporation. The increase in DNA is related to an increase in both protein content of healing wounds and of [^{14}C]leucine incorporation in protein. Then cell replication decreases. The parallel increase in DNA content of the healing wound and of SSAO activity seems to indicate that the replication is also an expression of fibroblast replication. In fact, the immunohistochemistry of the skin shows that this enzymic activity is mainly localized in fibroblasts, which is in full agreement with previous observations (Buffoni et al 1977).

Tissue-bound SSAO enzymes are described as being present primarily in vessels (Buffoni et al 1976; Lewinsohn et al 1978; Wibo et al 1980; Lewinsohn 1981) and localized mainly in smooth muscles (Hysmith & Boor 1987). A very modest activity is found in endothelial cells of the porcine aorta (Hysmith & Boor 1987). In skin, this enzymic activity appears to be mainly localized in fibroblasts and is therefore a good marker of the presence of these cells. During skin healing fibroblasts seem to appear on the 4th day, since there is a high correlation between the increase in DNA content and SSAO activity. The production of collagen starts later and is not yet complete after 11 days. This is indicated by the low levels of hydroxyproline which were still below the control values 11 days after surgery when, on the other hand, there is already a good degree of tissue reorganization. At this time SSAO activity is generally increased whereas the DNA content is close to the values of the normal skin. SSAO activity seems therefore expressed by fibroblasts when they start to produce the extracellular matrix.

The treatment of animals with methylprednisolone modifies the process by reducing the rate of re-epithelialization and increasing the rate of dermal collagen formation. Methylprednisolone at the dose used is an immunodepressing substance which reduces cell infiltration in the injured tissue and consequently the amount and quality of released growth factors. There is probably an increase in fibroblast growth factor in the skin of the animals treated with methylprednisolone and this might explain the observed positive relationship between the DNA content of the healing wound and the hydroxyproline content, whereas in the control animals there is a negative relationship.

The observation that during the healing process there was no variation in the peroxidase-like activity of albumin seems indicative of the small involvement of an inflammatory process, since the peroxidase-like activity of albumin is generally increased during inflammatory processes (Pirisino et al 1988).

Some data suggest that the biochemical aspects that accompany wound healing are directed by metabolism of the cellular infiltrate in the wound (Falcone & Caldwell 1990). The effect of methylprednisolone may support this hypothesis.

The use of biochemical markers such as those illustrated in this paper may permit monitoring of the process of skin healing and provide information correlating with the histological analysis, thus enabling this process, under the effect of different drugs, to be better understood.

Acknowledgements

We thank the MURST (Rome, Italy) and the Italian National Research Council for financial support of this work.

References

Bouchlier, M., Cavey, D., Kail, N., Hensby, C. (1990) Experimental models in skin pharmacology. Pharmacol. Rev. 42: 127–154

Buffoni, F., Marino, P., Pirisino, R. (1976) Partial purification of pig aorta amine oxidase. Ital. J. Biochem. 25: 191–203

Buffoni, F., Della Corte, L., Hope, D. B. (1977) Immunofluorescence histochemistry of porcine tissues using antibodies to pig plasma oxidase. Proc. R. Soc. Lond. 195: 417–423

Falcone, P. A., Caldwell, M. D. (1990) Wound metabolism. Clin. Plast. Surg. 17: 443–456

Hysmith, R. M., Boor, P. J. (1987) In vitro expression of benzylamine oxidase activity in cultured porcine smooth muscle cells. J. Cardiovasc. Pharmacol. 9: 668–674

Ignesti, G., Coppi, C. (1981) Determination of hydrogen peroxide with 2-nitro-5-mercaptobenzoic acid. Boll. Soc. Ital. Biol. Sper. 57: 2228–2234

Ignesti, G., Banchelli, G., Raimondi, L., Pirisino, R., Buffoni, F. (1992) Histaminase activity in rat lung and its comparison with intestinal mucosal diamine oxidase. Agents Actions 35: 192–199

Labarca, C., Paigen, K. (1980) A simple, rapid and sensitive DNA assay procedure. Anal. Biochem. 102: 344–352

Lewinsohn, R. (1981) Amine oxidase in human blood vessels and non-vascular smooth muscle. J. Pharm. Pharmacol. 33: 569–575

Lewinsohn, R., Heinrich Bohm, K., Glover, V., Sandler M. (1978) A benzylamine oxidase distinct from monoamine oxidase B. Widespread distribution in man and rat. Biochem. Pharmacol. 27: 1857–1863

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Lynch, S. E., Nixon, J. C., Colvin, R. B., Antoniades, H. N. (1987) Role of platelet-derived growth factor in wound healing: synergistic effects with other cofactors. Proc. Natl. Acad. Sci. USA 84: 7696–7700

Pirisino, R., Di Simplicio, P., Ignesti, G., Bianchi, G., Barbera, P. (1988) Sulphydryl groups and peroxidase-like activity of albumin as scanvenger of organic peroxides. Pharmacol. Res. Commun. 20: 545–552

Schilling, J. A. (1968) Wound healing. Physiol. Rev. 48: 374–423

Tallarida, R. J., Murray, R. B. (1984) Manual of Pharmacologic Calculations with Computer Programs. Springer-Verlag, New York, Heidelberg, Berlin

Wibo, M, Duong, A. T., Godfraind, T. (1980) Subcellular localization of semicarbazide-sensitive amine oxidase in rat aorta. Eur. J. Pharmacol. 112: 87–94

Wilkinson, G. N. G. (1961) Statistical estimations in enzyme kinetics. Biochem. J. 80: 324–332

Woessner, J. F. (1961) The determination of hydroxyproline in tissue and protein samples containing small proportion of this imino acid. Arch. Biochem. Biophys. 93: 440–447

Ziche, M., Banchelli, G., Caderni, G., Raimondi, L., Dolara, P., Buffoni, F. (1987) Copper-dependent amine oxidases in angiogenesis induced by prostaglandins (PGE1). Microvasc. Res. 34: 134–136