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Immunolocalization of heat shock proteins 27 and 47 during repair of induced oral ulcers

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Abstract: Heat shock proteins (Hsps) 27 and 47 are involved in the control of apoptosis, cell migration, and collagen synthesis. There is some understanding of the immunolocalization of these proteins during the repair process in skin and gastrointestinal mucosa, but their expressions in normal and injured oral mucosa are unknown. The aim of this study was to analyze the immunolocalization and intensity of these proteins in oral ulcers induced in rats and to compare these expression levels with those reported in skin and gastric mucosa. Ulcers were induced on the ventral surface of the tongues of rats. The rats were then euthanized at 0, 24, 48, 72, and 120 h. Hsp27 expression remained low in the first hours of repair, but was higher at 72 h, mainly in the migrating epithelium. Expression of Hsp47 was high at 48 h, mainly in fibroblasts, cells of the vascular wall, and basal keratinocytes of migrating epithelium. In the control group, expressions of these proteins were low, which indicates that these Hsps are constitutive proteins in oral mucosa. Expression levels were similar to those reported in the healing of skin lesions and gastric ulcer, suggesting a common mechanism of Hsp activation in the repair of these tissues. (J Oral Sci 52, 623-631, 2010)

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Introduction

The heat shock proteins (Hsps) are a family of highly conserved stress proteins that are expressed in bacteria, yeasts, and humans (1). They are a part of the chaperone group that is expressed intracellularly under normal conditions. Expression levels are increased during heat shock (2,3) and by other types of stress, such as infection, inflammation, recovery from anoxia, and glucose starvation (3). As cytoprotective chaperones, Hsps act in the conformational change of other proteins, the formation of protein multicomplexes, thermotolerance, and the prevention of protein aggregation. In addition, Hsps contribute to the correct synthesis of new proteins (4).

Hsp expression is increased during the normal reparative process and diminished during the chronic reparative process (2,3). Hypotheses regarding marked expression of Hsp during normal repair highlight the fact that, during healing, cells are exposed to a sequence of injuries, such as dehydration, alterations in the partial pressure of the oxygen and carbon dioxide, transient ischemia, and high production of reactive oxygen metabolites (5).

Among the Hsps, Hsp27 and Hsp47 may exert a special effect on the repair process due to their cytoprotective properties. Hsp27 belongs to the family of small heat shock proteins. These molecules resemble large oligomers of up to 800 KDa, and, in unstressed cells, Hsp27 levels are relatively low. During the stress response, the increase

in Hsp27 level is preceded by phosphorylation, which produces smaller molecules. In general, this induction is transient and the protein returns to baseline levels after removal of the stress agent. Hsp27 is also overexpressed during development and cell differentiation and has been observed in various embryological phases during skin formation (6) and craniofacial development (7). In addition, Hsp27 has been described as a molecular inhibitor of apoptosis, a property that contributes to its characteristic cytoprotection (8,9). Moreover, Hsp27 modulates fundamental activities during cell migration (10).

Hsp47 is present in the endoplasmic reticulum and is the main heat-induced glycoprotein associated with collagen in fibroblasts. It is overexpressed in tissues under certain pathophysiological conditions, such as fibrosis (11). In some cases of cellular stress, Hsp47 is released in the extracellular matrix linked to procollagen (12) and may contribute to the release of osteonectin in the extracellular matrix, thereby facilitating cell migration (13).

Because of the effects of Hsp27 on apoptosis and cell migration and the role of Hsp47 in collagen synthesis, these Hsps have been studied in the repair of skin and gastrointestinal mucosa. Studies have demonstrated that the immunohistochemical expressions of these proteins vary according to the repair phase and tissue type. Table 1 summarizes their main immunohistochemical patterns, as shown in experimental studies of skin and gastrointestinal mucosa. However, there are no such reports of Hsp27 and Hsp47 expression during the repair of oral mucosa, including the tongue. Therefore, we conducted an anatomical investigation of changes in the immunohistochemical expressions of Hsp27 and Hsp47 during the healing of ulcers on the tongues of rats. It is hoped that this analysis will increase understanding of the stress response in oral mucosa during the repair process. We limited our investigation to Hsp27 and Hsp47 because they are directly linked to cell migration and collagen synthesis, respectively. These mechanisms are crucial for mucosa regeneration, particularly for the migration of the epithelium and reconstruction of the lamina propria. In addition, to identify similarities and differences between oral mucosa, skin, and gastric mucosa, we compared the immunohistochemical results of this study with those of the studies listed in Table 1.

Table 1 Immunohistochemical expressions of Hsp27 and Hsp47 observed in studies of skin and gastric mucosal wounds

Authors	Site and lesion type	Analyzed Hsp	Tissue of focus	Expression of Hsp47 and Hsp27 on immunohistochemistry
Laplante et al. (5)	Mouse skin with or without induced wound	27, 60, 70, 90	Epidermis	Normal epidermis: suprabasal expression (Hsp27) Regenerating and migrating epithelia: higher in basal cells and lower in suprabasal cells (Hsp27)
Keagle et al. (14)	Rat skin with or without induced wound	32, 47, 70	Epidermis and dermis	Normal epidermis: no expression (Hsp47) Wound epidermis: high expression, mainly in basal cells (Hsp47) Normal dermis: low expression (Hsp47) Wound dermis: high expression in fibroblasts during 28 days (Hsp47)
Wang et al. (15)	Fetal and neonatal rat skin with or without induced wound	47	Epidermis and dermis	Normal fetal skin: expression observed in epidermal cell layer; none in subcutaneous tissue Wounded fetal skin: expression similar to that in normal skin Normal neonatal skin: equal to that in fetal skin Wounded neonatal skin: increased expression, both in epidermis and dermis
Guo et al. (16)	Rat gastric mucosa with induced ulcer	32, 47, 70	Entire gastric mucosa	Distant from ulcer and normal mucosa: high expression in connective tissue (Hsp47) Adjacent to ulcer: high expression in fibroblasts and endothelial cells of lamina propria, submucosa, serosa layer, and muscularis (Hsp47)
Ebert et al. (17)	Biopsies of human gastric ulcer; mouse induced gastric ulcer	27	Entire gastric mucosa	Adjacent to ulcer: high expression in endothelial cells, fibroblasts, smooth muscle fibers in ulcer base, in epithelium (granular layer), and at base of fundic glands Distant from ulcer: none in tissue as a whole

Hsp: heat shock protein

Materials and Methods

This study was approved by the Ethics Committee for Experimental Research of the School of Dentistry, University of São Paulo, in accordance with the guidelines of the Brazilian Council on Animal Care and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

Experimental groups

Thirty adult female Wistar rats (*Rattus norvegicus*) weighing about 200 g were divided randomly into an ulcer group comprising 25 animals with induced ulcers on the ventral surface of the tongue (5 animals per experimental time point) and a control group comprising 5 animals without ulcers.

During the experiment, the animals were kept under a clear-dark cycle at a controlled temperature (21°C). The rats were euthanized with an overdose of anesthetic (ketamine and xylazine). The ulcer group animals were euthanized at 0, 24, 48, 72, and 120 h, and the control group animals were euthanized at 120 h.

Induction of ulcers on rat tongues

Ulcers were induced on the ventral surface of the tongue, using a protocol adapted from Fujisawa et al. (18). The animals were anesthetized with an intraperitoneal injection of ketamine (Dopalen®, Vetbrands, Paulinia, SP, Brazil) and xylazine (Anasedan®, Vetbrands, Paulinia, SP, Brazil) (0.1 ml/kg and 0.01 ml/kg, respectively). While the tongue was gently pulled outside of the mouth, a filter paper (5 mm × 5 mm) containing 20 μl of a 50% acetic acid solution was applied to the ventral surface of the tongue for 60 seconds. The animals were kept in individual cages and given commercial feed and water *ad libitum*.

Immunohistochemical technique

After euthanization, the tongue was excised, immediately fixed in neutral buffered 10% formaldehyde solution, and embedded in paraffin. The streptavidin-biotin-peroxidase technique was used for tests with monoclonal and polyclonal antibodies against Hsp27 and Hsp47 (Abcam, Cambridge, MA, USA), respectively. Three-micrometer-thick sections were stretched on glass slides treated with 3-aminopropyltriethoxysilane. These were dewaxed and rehydrated in a descending series of alcohols. Antigen retrieval by microwave and citrate buffer (pH 6.0) was performed for Hsp27 antibody, using the procedure specified by the antibody manufacturer and described in the literature (19). Slides were subjected to endogenous tissue peroxidase blocking. Incubation was performed with the primary antibody at a dilution of 4 μg/ml for Hsp27 and 1:1000 for Hsp47. The samples were then incubated with a biotinylated swine-anti-rabbit/goat antibody, as well as a streptavidin-biotin-peroxidase conjugate (LSAB System, Dako®, Carpenteria, CA, USA) for 30 min each. The reaction was then revealed by diaminobenzidine (Dako®), and the sections were colored by Mayer hematoxylin, dehydrated in an increasing series of alcohols, immersed in xylol, and mounted in resin for conventional light microscopy. For the negative control, sections were incubated in a buffer without primary antibody.

As an additional immunohistochemical test, immunohistochemical reactions against PCNA (proliferating cell nuclear antigen) and cytokeratin 14 were performed to observe the rate of cell proliferation and basal/suprabasal keratinocyte origin, respectively, in the migrating epithelium. Representative microscopic sections with visible migrating epithelium underwent the same, abovementioned process of dewaxing and dehydration. Primary antibodies against PCNA (1:100; Dako®) and cytokeratin 14 (1:500; NeoMarkers®, Fremond, CA, USA) were used after antigen retrieval (citric acid in water bath for 30 min and 45 min for PCNA and cytokeratin 14, respectively). Incubation and reaction revelation of secondary antibodies were the same as those described for Hsp.

Immunolocalization and semiquantitative analysis

A semiquantitative analysis was performed by two observers, both of whom examined the histological sections labeled for Hsp27 and Hsp47 without knowledge of the experimental time point. The observers classified the results of the immunohistochemical test as: 0, no expression; 1, low expression; 2, low-to-moderate expression; 3, moderate-to-high expression; 4, high expression. The regions analyzed were the layers of the preexisting and migrating epithelium, fibroblasts, endothelium, skeletal muscle fibers, extracellular matrix, and inflammatory cells in the region of the ulcer.

Results

Figure 1 shows the clinical appearance of the ulcers at all experimental time points. The ventral surface of the tongue had completely regenerated at 120 h.

Table 2 shows the intensity of the immunohistochemical expression of Hsp27 and Hsp47. In general, Hsp27 and Hsp47 expressions had a granular appearance in the cytoplasm of cells in both the control and ulcer groups.

Hsp27

In the control group, there was moderate-to-high immunohistochemical expression of Hsp27 in the middle and superficial layers of preexisting epithelium, as well

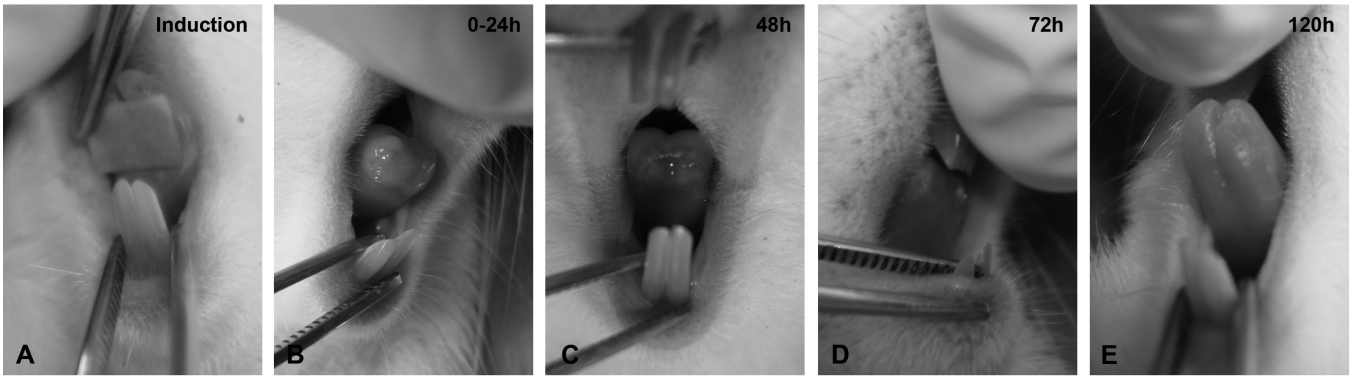

Fig. 1 Clinical appearance of ulcers. A: Filter paper with acetic acid applied to ventral surface of tongue. B: Necrotic surface, ulceration, and intense edema. C: Initial reepithelization, granulation tissue formation, and absorption of necrotic tissue. D: Mature granulation tissue and no clinical signs of ulceration. D: Complete repair with total mucosal regeneration.

Table 2 Median values in semiquantitative analysis of Hsp27 and Hsp47 immunohistochemical expression in the control (C) and ulcer (U) groups

Site of analysis	Hsp27						Hsp47					
	C	U – Duration of healing, h					C	U – Duration of healing, h				
		0	24	48	72	120		0	24	48	72	120
Granular layer – normal, preexisting epithelium	3	1	1	2	2	3	0	0	0	0	0	0
Suprabasal layer – normal, preexisting epithelium	2	1	1	1	2	2	0	0	0	0	0	0
Basal layer – normal, preexisting epithelium	0	0	0	0	2	0	0	1	0	0	0	0
Superficial cells – migrating epithelium	×	×	1	2	2	3	×	×	1	0	0	0
Suprabasal cells – migrating epithelium	×	×	1	1	2	1	×	×	2	2	1	0
Basal cells – migrating epithelium	×	×	1	2	2	0	×	×	2	3	3	1
Fibroblasts	1	0	0	1	1	0	1	2	3	4	3	3
Cells of vascular wall	1	1	1	2	2	1	0	2	2	3	3	1
Inflammatory cells	0	1	1	1	1	0	0	0	0	0	0	0
Extracellular matrix	0	2	1	2	1	0	0	2	2	2	1	0
Skeletal muscle fibers	3	4	3	3	3	3	0	0	0	0	0	0

×: absence of structure, 0: no expression, 1: low expression, 2: low-to-moderate expression, 3: moderate-to-high expression, 4: high expression, Hsp: heat shock protein

as in skeletal muscle fibers (Fig. 2A). Expression was low in fibroblasts and cells of the vascular wall. There was no expression in the extracellular matrix.

In the ulcer group, the preexisting epithelium at the ulcer border exhibited low expression of Hsp27 at 0 h (Fig. 2B). At 24 h, the migrating epithelium exhibited low expression, mainly in superficial layers (Fig. 2C). From 48 h to 120 h low-to-moderate expression was observed

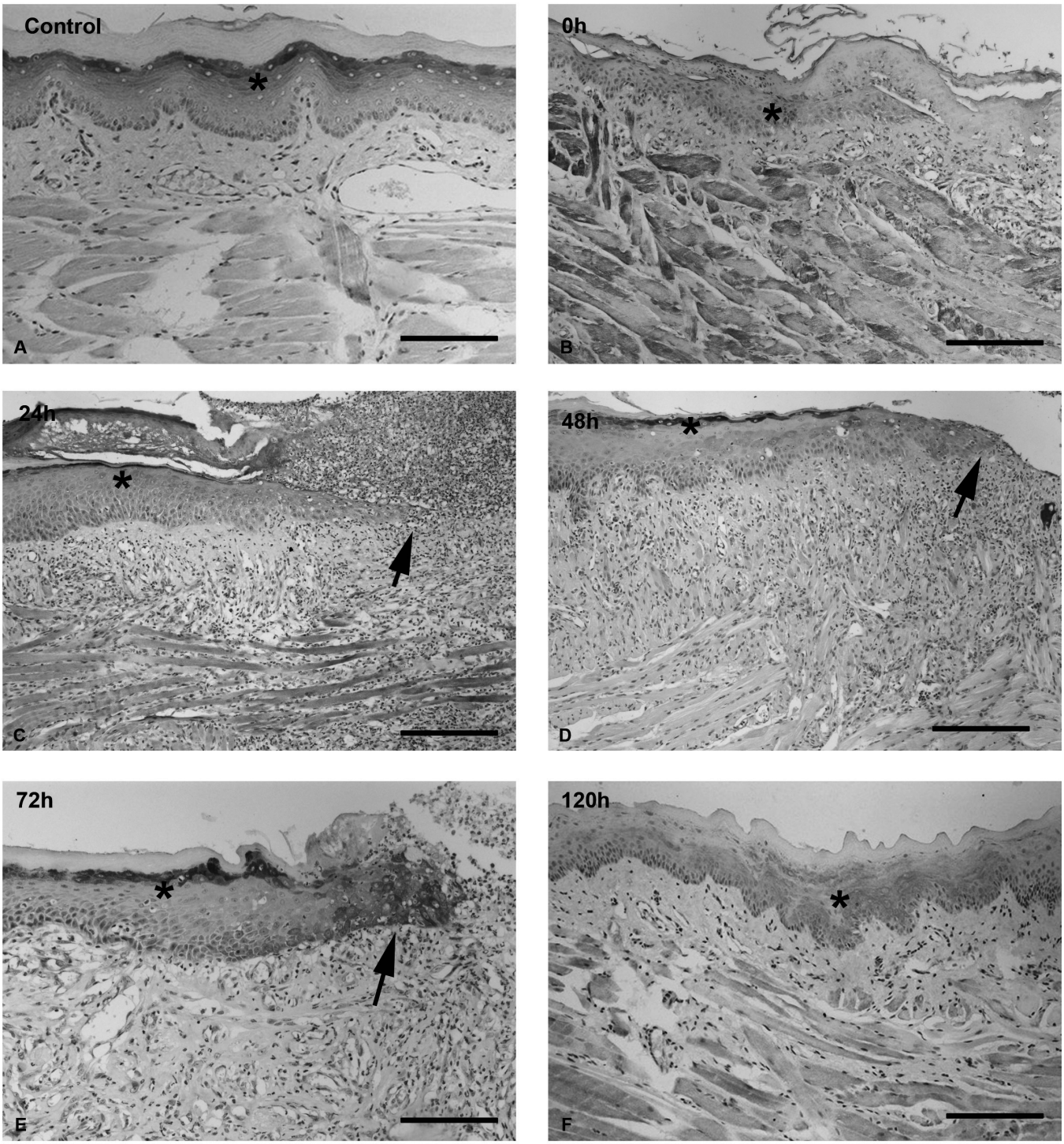

Fig. 2 Immunohistochemical expression of Hsp27 in streptavidin-biotin tests (Original magnification: A and E, × 200, bar = 100 μm; B through F, × 100, bar = 200 μm). A: Strong positivity in epithelial granular layer (*), epithelial suprabasal layer, and in muscle fibers; no expression in basal layer of epithelium. B: Low expression in granular layer of epithelium adjacent to ulcer (*), high expression in muscle fibers, and no expression in other visible structures. C: Positivity in migrating epithelium in superficial layers and in some basal keratinocytes (arrow); expression also observed in granular layer of preexisting epithelium (*), as well as in vascular wall. D: Moderate positivity in migrating epithelium (arrow), including some keratinocytes of basal layer, and in superficial layers of preexisting epithelium (*); low expression in vascular wall of lamina propria. E: All layers of migrating epithelium show moderate positivity (arrow); similar expression observed in vascular wall and in regenerating muscle fibers. The preexisting epithelium shows positivity in superficial layers (*). F: Complete ulcer healing; Hsp27 expression is similar to that of control in both the epithelium (*) and lamina propria/submucosa.

in all epithelial layers of the migrating epithelium and in the granular and suprabasal layers of preexisting epithelium (Figs. 2D to 2F). Expression was low in the lamina propria, fibroblasts, and plasma cells; endothelial cells showed moderate expression at 48 h and 72 h. At these experimental time points, extracellular expression was also observed in edema fluid. Muscle fibers showed high expression levels at all time points. At 120 h, when repair was complete, the levels of Hsp27 expression in these tissue elements were similar to those observed in the control group (Fig. 2F).

Hsp47

In the control group, Hsp47 was expressed only in fibroblasts in the lamina propria adjacent to the epithelium (Fig. 3A).

In the ulcer group, Hsp47 was not expressed in preexisting epithelium at most time points. At 0 h, only fibroblasts exhibited expression (Fig. 3B). However, in the migrating epithelium, high expression levels were observed, mainly in basal keratinocytes, from 24 h to 72 h (Figs. 3C to 3E). The highest intensity was observed at 48 h in fibroblasts and endothelial cells in granulation tissue (Fig. 3D). Extracellular expression was observed in these phases (Figs. 3B, 3C, 3D). After the oral mucosa had completely regenerated, high Hsp47 expression was present only in fibroblasts; low expression was observed in a few basal keratinocytes at this time point (Fig. 3F).

The specialized epithelium of the tongue dorsum also exhibited expression of Hsp27 and Hsp47; the expression patterns were similar to those observed in ventral epithelium (data not shown).

Figure 4 shows sections at 24 h labeled with anti-cytokeratin 14 and anti-PCNA. Cytokeratin 14 positivity in the thin, discontinuous epithelium covering the ulcer confirms that these cells are migrating basal keratinocytes. PCNA positivity in the same region indicates high proliferation of these cells and suggests that expressions of Hsp27 and Hsp 47 in the migrating epithelium at 24 h are associated with high proliferation of undifferentiated (basal) keratinocytes.

Discussion

We investigated the immunohistochemical expression of Hsp27 and Hsp47 during the healing of ulcers induced on the tongues of rats. In addition to the experimental and immunohistochemical tests, a literature review was conducted to compare our immunohistochemical findings with those observed in skin and gastric mucosa. Experimental studies with similar, induced lesions (such as wound incisions and acid-induced ulcers) were chosen. Studies indicate that Hsp27 is a constitutive protein in the epidermis and is expressed in the suprabasal layers under normal conditions (5). It is not, however, expressed in normal gastric mucosa (17). Hsp47 is a constitutive protein in the connective tissue of these sites (14,16) and in the epidermal layer of fetal and neonatal rats (15). In the normal mucosa of the tongue, the present experiment showed immunohistochemical expression patterns similar to those observed in skin, i.e., Hsp27 and Hsp47 are constitutive proteins in the suprabasal layer of the oral epithelium and the lamina propria, respectively.

Our examination of the expression of inducible Hsps in the ulcer group revealed differences in Hsp27 and Hsp47 expression, as compared with normal mucosa, at all time points, i.e., from 0 h to 120 h. Low expression of Hsp27 was observed until 72 h in the epithelial margins of the ulcer and in keratinocytes relatively distant from superficial necrotic tissue, which suggests that the acid-induced injury did not increase synthesis of this protein; on the contrary, its expression decreased, which may be due to cell limitations in Hsp synthesis. A similar result regarding the epithelial borders of gastric ulcers was observed in a study of Hsp70 and Hsp32 (16). However, in the present study, the migrating epithelium showed high Hsp27 expression, which suggests that there are cell-type specificities for Hsp27 production during healing. These specificities are restricted to proliferating keratinocytes, which require greater control of apoptosis and cytoskeleton contraction in migration during epithelium regeneration. The high expression of Hsp27 in the migrating epithelium was similar to that observed in skin lesions (5) and gastric ulcer (17). When regeneration of the epithelium was complete, Hsp27 positivity was restricted to the suprabasal layers, as in normal mucosa. Hsp27 expression was consistent with that observed in cells with high differentiation and low proliferation status (5), which highlights the additional functions of Hsp27 with respect to cell differentiation control (6).

Changes in Hsp27 expression were also observed in the vascular wall, namely, increased intensity was noted during granulation tissue formation. This finding is consistent with the fact that Hsp27 regulates the migration of endothelial cells (20), a process that is particularly intense during angiogenesis. Similar findings were observed in gastric ulcer repair (17).

In relation to inducible Hsp47, we observed increased immunohistochemical expression at 48 h. Some reports noted late expression of Hsp47 during healing, suggesting that the role of Hsp47 in repair is directly linked to granulation tissue formation, collagen deposition, and the remodeling phase of the repair process (16,21). In the present study, Hsp47 expression decreased to its initial

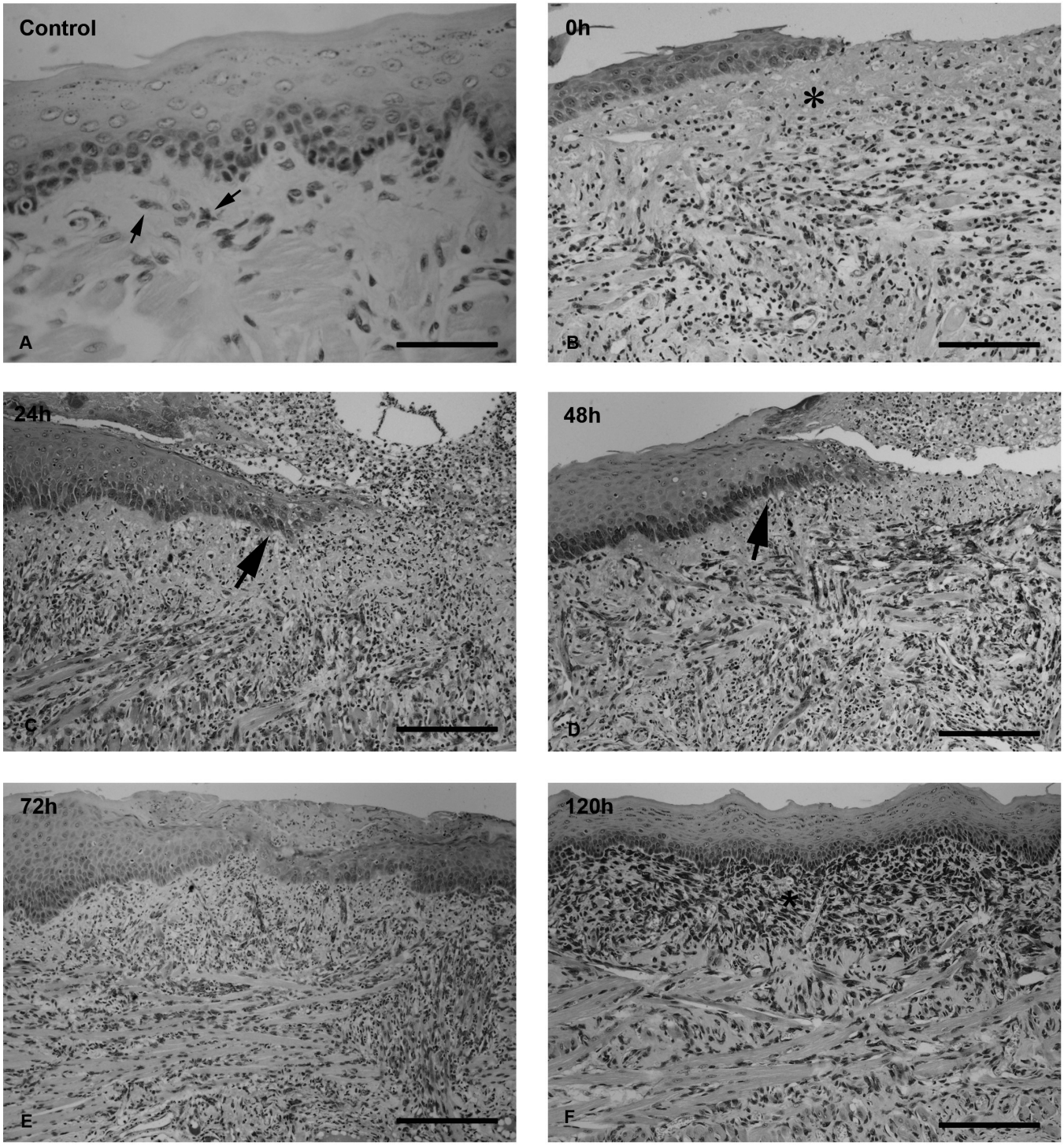

Fig. 3 Immunohistochemical expression of Hsp47 in streptavidin-biotin tests (Original magnification: A, × 400, bar = 50 μm; B to F, × 100, bar = 200 μm). A: Positivity only in fibroblasts adjacent to epithelium (arrows); no Hsp47 expression in any layer of epithelium. B: Low expression in basal keratinocytes in preexisting epithelium adjacent to ulcer. Low expression is also observed in interstitial space of connective tissue (*). Expression in fibroblasts is more intense during this phase. C: Migrating epithelium with moderate expression in basal keratinocytes in long section of epithelium (arrow), and multiple fibroblasts with strong positivity in basal region of ulcer. D: Basal keratinocytes exhibit strong Hsp47 expression in migrating epithelium (arrow). Fibroblasts of granulation tissue also show high expression, as do cells in vascular wall. E: Regeneration of epithelium is almost complete; low-to-moderate immunohistochemical expression is present in basal and suprabasal layers; fibroblasts and endothelial cells show high expression in granulation tissue. F: Fibroblasts of granulation tissue adjacent to regenerating epithelium exhibit strong positivity (*); no expression in keratinocytes in superficial layers; low expression in some basal keratinocytes.

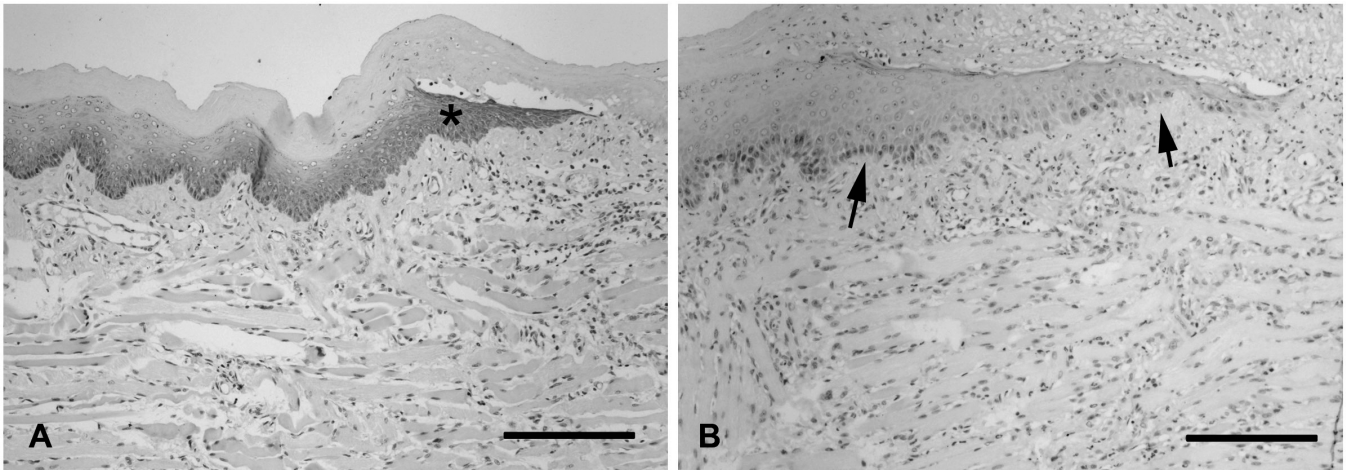

Fig. 4 Immunohistochemical expression of cytokeratin 14 and PCNA in streptavidin-biotin tests (Original magnification: A, × 100, bar = 200 μm; B, × 200, bar = 100 μm). A: Moderate-to-high expression of cytokeratin 14 in basal keratinocytes at ulcer border and keratinocytes in thin epithelium at ulcer surface (*). B: Basal keratinocytes exhibit strong PCNA immunolabeling, both in epithelium at ulcer border and in some keratinocytes in migrating epithelium at ulcer surface (arrows). Both sections were obtained from the same 24-h specimen shown in Fig. 2C.

intensity at 120 h, when complete regeneration of the lamina propria was observed, without intense collagen deposition. We believe that the remodeling phase of connective tissue in the ulcer group was transient because of the limited damage to the lamina propria. This fact, along with the presence of discrete granulation tissue, might explain the reduction in Hsp47 expression at 120 h. Other researchers (14) observed an increase in Hsp47 on days 1 and 2 of repair, which indicates that this protein has a role in early signaling during collagen deposition and might thus be a useful indicator of collagenic synthesis.

Hsp47 expression was observed in the basal keratinocytes of the migrating epithelium, which was similar to findings in skin lesions (14). Hsp47 is absent in normal oral epithelium; thus, it appears that regenerating oral epithelium is able to regain Hsp47 expression similar to that present in the epidermal layer of fetal and neonatal rats (15). Although there has been no investigation of Hsp47 expression in the oral mucosa of fetal or neonatal rats, studies involving other Hsps during the development of tooth germs demonstrated low expression of these proteins in the oral mucosa of rat embryos (22). In cultures of keratinocytes exposed to heat shock, Hsp47 expression was associated with osteonectin expression, suggesting that this Hsp is involved in extracellular matrix production by keratinocytes and in migration during healing (13).

We also observed substantial immunohistochemical expression of Hsp27 and Hsp47 in the extracellular compartment. Hsp27 can be secreted by cells and act as an anti-inflammatory (23). Hsp47 can be released to the extracellular matrix, in conjunction with procollagen and osteonectin secretion (12). We detected Hsp27 mainly in edema fluid; Hsp47 was primarily observed in the extracellular matrix of granulation tissue. The presence of both Hsps could also interfere with the healing process. Additional studies are necessary to determine the role of extracellular Hsps in oral tissue repair.

A limitation of the present study was the quantification of the Hsps. It is believed that immunohistochemical intensity is related to different functions of these proteins in the healing process. Therefore, quantitative studies are necessary to elucidate Hsp oscillations in the different phases of wound healing in oral mucosa.

In conclusion, we detected immunohistochemical expression of Hsp27 and Hsp47 in normal tongue mucosa, which suggests that these proteins are constitutive in this tissue, as is the case in skin and gastric mucosa. Inducible Hsp27 also had an immunohistochemical pattern similar to that observed in the healing of skin and gastric mucosa; however, some chronological differences were observed in inducible Hsp47 expression in oral ulcers, when expression levels were compared with those in skin and gastric wounds. The similarities of inducible Hsps in tongue mucosa, skin, and gastric mucosa suggest a common mechanism underlying Hsp activation in the repair of these tissues.

References

1. Kregel KC (2002) Heat shock proteins: modifying factors in physiological stress responses and acquired

thermotolerance. J Appl Physiol 92, 2177-2186.
2. Kovalchin JT, Wang R, Wagh MS, Azoulay J, Sanders M, Chandawarkar RY (2006) In vivo delivery of heat shock protein 70 accelerates wound healing by up-regulating macrophage-mediated phagocytosis. Wound Repair Regen 14, 129-137.
3. Tsan MF, Gao B (2004) Heat shock protein and innate immunity. Cell Mol Immunol 1, 274-279.
4. Hartl FU, Hayer-Hartl M (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 295, 1852-1858.
5. Laplante AF, Moulin V, Auger FA, Landry J, Li H, Morrow G, Tanguay RM, Germain L (1998) Expression of heat shock proteins in mouse skin during wound healing. J Histochem Cytochem 46, 1291-1301.
6. Monastirli A, Vourekas A, Badavanis G, Pasmatzi E, Sagriotis A, Drainas D, Pavlidou D, Georgiou S, Sakkis T, Mantagos S, Kourounis G, Varakis J, Stamatiou G, Tsambaos D (2005) Hsp27 expression coincides with epidermal stratification during human epidermal morphogenesis. Acta Derm Venereol 85, 389-393.
7. Leonardi R, Barbato E, Paganelli C, Lo Muzio L (2004) Immunolocalization of heat shock protein 27 in developing jaw bones and tooth germs of human fetuses. Calcif Tissue Int 75, 509-516.
8. Bruey JM, Ducasse C, Bonniaud P, Ravagnan L, Susin SA, Diaz-Latoud C, Gurbuxani S, Arrigo AP, Kroemer G, Solary E, Garrido C (2000) Hsp27 negatively regulates cell death by interacting with cytochrome c. Nat Cell Biol 2, 645-652.
9. Concannon CG, Gorman AM, Samali A (2003) On the role of Hsp27 in regulating apoptosis. Apoptosis 8, 61-70.
10. Hirano S, Shelden EA, Gilmont RR (2004) HSP27 regulates fibroblast adhesion, motility, and matrix contraction. Cell Stress Chaperones 9, 29-37.
11. Nagata K, Hosokawa N (1996) Regulation and function of collagen-specific molecular chaperone, HSP47. Cell Struct Funct 21, 425-430.
12. Nagata K (1998) Expression and function of heat shock protein 47: a collagen-specific molecular chaperone in the endoplasmic reticulum. Matrix Biol 16, 379-386.
13. Kudo H, Hirayoshi K, Kitagawa Y, Imamura S, Nagata K (1994) Two collagen-binding proteins, osteonectin and HSP47, are coordinately induced in transformed keratinocytes by heat and other stresses. Exp Cell Res 212, 219-224.
14. Keagle JN, Welch WJ, Young DM (2001) Expression of heat shock proteins in a linear rodent wound. Wound Repair Regen 9, 378-385.
15. Wang ZL, Inokuchi T, Ikeda H, Baba TT, Uehara M, Kamasaki N, Sano K, Nemoto TK, Taguchi T (2002) Collagen-binding heat shock protein HSP47 expression during healing of fetal skin wounds. Int J Oral Maxillofac Surg 31,179-184.
16. Guo JS, Cho CH, Wang JY, Koo MW (2002) Expression and immunolocalization of heat shock proteins in the healing of gastric ulcers in rats. Scand J Gastroenterol 37, 17-22.
17. Ebert MP, Schäfer C, Chen J, Hoffmann J, Gu P, Kubisch C, Carl-McGrath S, Treiber G, Malfertheiner P, Röcken C (2005) Protective role of heat shock protein 27 in gastric mucosal injury. J Pathol 207, 177-184.
18. Fujisawa K, Miyamoto Y, Nagayama M (2003) Basic fibroblast growth factor and epidermal growth factor reverse impaired ulcer healing of the rabbit oral mucosa. J Oral Pathol Med 32, 358-366.
19. Kase S, Parikh JG, Rao NA (2009) Expression of heat shock protein 27 and alpha-crystallins in human retinoblastoma after chemoreduction. Br J Ophthalmol 93, 541-544.
20. Landry J, Huot J (1995) Modulation of actin dynamics during stress and physiological stimulation by a signaling pathway involving p38 MAP kinase and heat-shock protein 27. Biochem Cell Biol 73, 703-707.
21. Tsukimi Y, Nakai H, Itoh S, Amagase K, Okabe S (2001) Involvement of heat shock proteins in the healing of acetic acid-induced gastric ulcers in rats. J Physiol Pharmacol 52, 391-406.
22. Wada H, Kobayashi I, Yamaza H, Matsuo K, Kiyoshima T, Akhtar M, Sakai T, Koyano K, Sakai H (2002) In situ expression of heat shock proteins, Hsc73, Hsj2 and Hsp86 in the developing tooth germ of mouse lower first molar. Histochem J 34, 105-109.
23. Calderwood SK, Mambula SS, Gray PJ Jr, Theriault JR (2007) Extracellular heat shock proteins in cell signaling. FEBS Lett 581, 3689-3694.