MORPHOLOGY AND PATHOMORPHOLOGY

Use of Bilidase for the Treatment of Experimental Hypertrophic Postburn Cicatrices

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The efficiency of bilidase (hyaluronidase preparation) was studied in guinea pigs with experimental postburn cicatrices. Bilidase promoted normalization of the structure and histochemical picture of new cicatricial tissue. The drug can be used for the treatment of hypertrophic postburn cicatrices.

Key Words: *hyaluronidase; bilidase; hyaluronic acid; hypertrophic cicatrix*

Skin wounds are usually healed with slight cicatrices, even if the dermis is involved. However, in some cases pathological (mainly hypertrophic) cicatrices are formed. Complications presenting as cicatricial transformations often develop after II and III degree burns.

These transformations, in addition to esthetically undesirable effects, often cause complex functional disorders. The cicatrices are usually resistant to treatment. The majority of treatment methods, such as surgical intervention, pressure treatment, laser therapy, drug therapy, *etc.*, are not universally effective, and hypertrophic cicatrices usually appear again. The search for new conservative methods for the prevention and treatment of hypertrophic cicatrices is a serious problem of modern medicine [5].

Connective tissue diseases, specifically, the formation of hypertrophic posttraumatic cicatrices, are associated with accumulation of acid glucosaminoglycans (particularly hyaluronic acid) in collagen fibers. High content of hyaluronic acid promotes higher strength and strain of cicatrices in comparison with normal skin. Hyaluronidase, an enzyme catalyzing depolymerization of hyaluronic acid, can be an effective means for the treatment of this condition [3].

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The methods of isolation and purification of bacterial hyaluronidase were developed at the Laboratory of Bacterial Biochemistry, G. Eliava Institute of Bacteriophages, Microbiology, and Virology. Bilidase is a new drug created on the basis of highly purified and highly active bacterial hyaluronidase. Preclinical and clinical trials of the drugs were successful and now bilidase is widely used in Georgian clinics [10].

We studied the possibility of using bilidase for the treatment of hypertrophic postburn cicatrices.

MATERIALS AND METHODS

Burns of IIIA and B degree were inflicted under narcosis to 50 guinea pigs (230-250 g).

For experimental reproduction of postburn cicatricial transformations, thermal injuries were induced by contact method with measurement of subcutaneous temperature by the method of Kichetov.

The treatment of experimental wounds was started 1 month after primary healing. In group 1 animals (n=40) bilidase was daily injected into the area of the cicatrix for 2 weeks. Some of animals (group 2, n=20) received a repeated course after 1 month. Group 3 animals (n=10) received no treatment.

The material for histological and histochemical studies was collected from different sites of the

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cicatrix (5-6 fragments). Tissue fragments were fixed in 10% neutral formalin and Carnoy fluid and embedded in paraffin. Serial sections were stained with hematoxylin and eosin. Argirophilic substance was detected by the method of Gomori, elastic fibers were stained with Weigert resorcin-fuchsine. Glycogen and neutral glycoproteins were detected by Shabadash method, acid glycosaminoglycans by Alcian Blue staining. Some preparations were treated with appropriate enzymes for identification of acid glycosaminoglycans [4,9].

RESULTS

Untreated cicatrices had clear-cut borders and sometimes were as if "hanging over" the adjacent skin; they had glossy purple surface and elastic consistency. In some sites the cicatrices were covered with crusts; hairy bundles were sometimes seen at the edges.

Bilidase therapy led to an appreciable shrinking of the cicatrix. Its consistency changed, the cicatrix became softer, and, if pressed, did not grow pale; pigmentation and hairy bundle appeared on the entire cicatrix.

After repeated course of bilidase therapy the site of the cicatrix almost did not differ from normal skin; there was just slight pigmentation and hairs were scantier compared to adjacent skin.

The morphological basis of the cicatrix is excessively growing connective tissue with numerous atypical fibroblasts. The cicatrix is presented by coarse, often fragmented bundles of collagen fibers without definite orientation. Hyalinosis and hemorrhagic foci are often seen. Appreciable volumes of new connective tissue (growth zones or bundles) are characteristic of some sites of the cicatrix. Transition into deeper layers of the cicatrix is gradual; the lower layers are presented by more mature, often hyalinized fibrous tissue. Narrow dermal layer, retained at some sites, contains solitary capillaries with stenosed lumen; sometimes blood vessels are completely obliterated (Fig. 1, *a*).

Acanthosis type growth of the epidermis is observed in some areas of the cicatrix. The skin is involved through its entire depth, with few more or less intact deep layers with skin appendages retained somewhere in the derma. The dermal structure is heterogeneous, in some visual fields the reticular layer is presented by coarse fragmented bundles of collagen fibers. Small foci with partially retained papilla of the derma are seen along the edge of the cicatrix. Desquamation and separation of dermal layers were sometimes seen. Fibrous base of surface papillary layer was in a state of disorientation and destruction. Connective tissue fibers were swollen, some of them necrotic (Fig. 1, *b*).

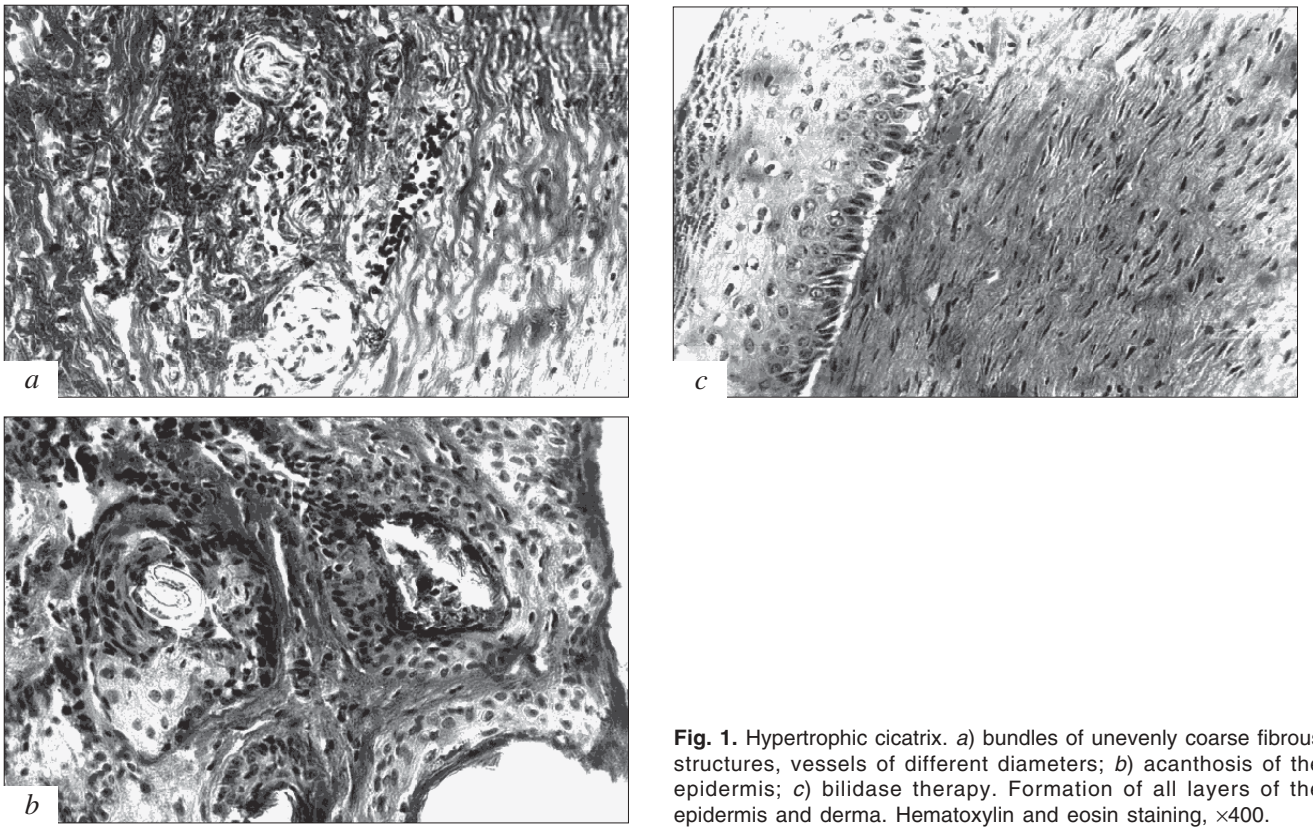

Fig. 1. Hypertrophic cicatrix. *a*) bundles of unevenly coarse fibrous structures, vessels of different diameters; *b*) acanthosis of the epidermis; *c*) bilidase therapy. Formation of all layers of the epidermis and derma. Hematoxylin and eosin staining, ×400.

Histochemical study showed acid glycosaminoglycans, argirophilic substance, and neutral glycoproteins in the cicatrices. Numerous acid glycosaminoglycans were detected in the fibers around vessels, presenting as amorphous substance.

Injection of bilidase into the cicatrix had an appreciable pharmacotherapeutic effect. Bilidase promoted normalization of transformed tissue. Formation and maturation of the derma and its epithelialization were noted. In contrast to untreated cicatrices, bilidase treatment led to the appearance of cells of the pricky and granular layers of the epithelium. Basal layer cells were best seen. The count of fibroblasts in cicatricial tissue decreased: elongated fibroblasts were situated between bundles of fibrous structures and were oriented along fibrillar structures of the connective tissue. Subepithelial layer fibers were regularly arranged (Fig. 1, *c*).

After repeated course of hyaluronidase therapy all layers of the epidermis were clearly defined, the structure and histochemical picture of the derma were almost completely restored. Fibrous structures were arranged parallel to each other; numerous new capillaries were seen.

Histochemical examination showed that the content of acid glycosaminoglycans decreased after bilidase therapy, the content of neutral glycoproteins increased, and glycogen appeared in basal cells of the epidermis.

Skin injuries are characterized by accumulation of collagen-rich fibrous tissue with high content of proteoglycans (mainly hyaluronic acid). Complications presenting as cicatricial degeneration, mainly hypertrophic cicatrices, often develop after II and III degree burns. Hypertrophic cicatrices are unique fibroproliferative lesions of the derma developing after injuries, burns, operations, and inflammatory processes. These are elevated erythematous itching lesions, usually remaining in the wound and often linked with the healing tissue contractures. These cicatrices are usually resistant to the known methods of treatment [7,11].

Hyaluronidase, an enzyme polymerizing hyaluronic acid, the main connective tissue substance, and thus decreasing the viscosity of interstitial fluid and increasing tissue permeability.

Hence, bilidase had a good therapeutic effect, promoting normalization of the structure and histochemical picture of cicatricial degenerative tissue. Presumably, the use of hyaluronic preparations in the treatment of hypertrophic cicatrices will promote the penetration of endogenous enzymes into cicatricial tissue and along with cleavage of hyaluronic acid will accelerate cleavage of excessive collagen and proteoglycan aggregations. Our experiments demonstrated good prospects of using bilidase for the treatment of hypertrophic cicatrices of different origin.

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