EFFECT OF CYSTAMINE (2-AMINOETHANETHIOL) ON MORPHOLOGY OF GUNSHOT WOUND HEALING (LIGHT-OPTICAL AND ELECTRON-MICROSCOPIC STUDY)

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UDC 617-001.45-085.849.1.015.25-036.8:617-001.45-003.9

KEY WORDS: cystamine (2-aminoethanethiol), gunshot wounds, healing.

An intensive search for new substances which will shorten the time of wound healing still continues [1, 4, 5, 8-10]. Compounds of the aminothiol group and, in particular, cystamine (2-aminoethanethiol), have been shown to have a marked stimulating action on repair process. However, experimental and clinical data on acceleration of wound healing under the influence of cystamine are very scanty [6, 7, 9] and there has been no attempt to study on account of which phases the duration of wound healing is shortened by cystamine, or the ultrastructural and enzyme-histological changes that take place in the wound tissues under these circumstances.

The aim of this investigation was to study the effect of cystamine on the times and phases of wound healing and on the ultrastructure and enzyme-histology of the tissues of gunshot wounds.

EXPERIMENTAL METHOD

Experiments were carried out on 24 rabbits of both sexes weighing 2-2.5 kg. Under thiopental anesthesia, a perforating gunshot wound of the soft tissues of the thigh was inflicted on the animals by a standard technique by a shot from a 5.6 mm pistol at a distance of 10 cm. Primary surgical treatment (PST) of the wound was carried out 24 h later. For 14 days the experimental animals were treated with cystamine by intravenous injection in a dose of 0.25 mg/kg body weight, whereas control animals received distilled water.

The rabbits were killed by air embolism 1, 3, 5, 7, 9, 14, 21, and 28 days after PST (three animals each time) and material was taken from the wound for morphological investigation. Pieces of tissue from the wound region and from macroscopically unchanged areas adjacent to it for histochemical reactions were stabilized in liquid nitrogen. Activity of alkaline and acid phosphomonoesterases (AlP and AcP respectively) was determined in freshly frozen sections cut on a freezing microtome by Burstone's and azo-coupling methods, and NADPH- and NADH-diaphorase activity was determined by modified methods of Nachlas, Walker, and Seligman. Histological survey preparations were obtained on freshly frozen sections cut in a freezing microtome and stained with hematoxylin and eosin and with azure II. Pieces of tissue for electron-microscopic investigation were fixed by Caulfield's method and embedded in Epon-812; ultrathin sections were examined in the JEM-100CX electron microscope. Preliminary analysis of the material was carried out on semithin sections [2]. Details of the technique of obtaining and processing the material were described previously [3].

EXPERIMENTAL RESULTS

During the first 3 days a mild degree of serofibrinous edema and moderate infiltration with polymorphonuclear leukocytes (polymorphs) were observed in the wound tissues of the experimental animals. The inflammatory changes continued during the first week and were localized mainly in the surface areas of the wound. Compared with control rabbits, AlP activity

Research Laboratory of Electron Microscopy and Histochemistry, S. M. Kirov Military Medical Academy, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR I. S. Kolesnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 12, pp. 723-727, December, 1984. Original article submitted November 26, 1983.

0007-4888/84/9812-1713$08.50

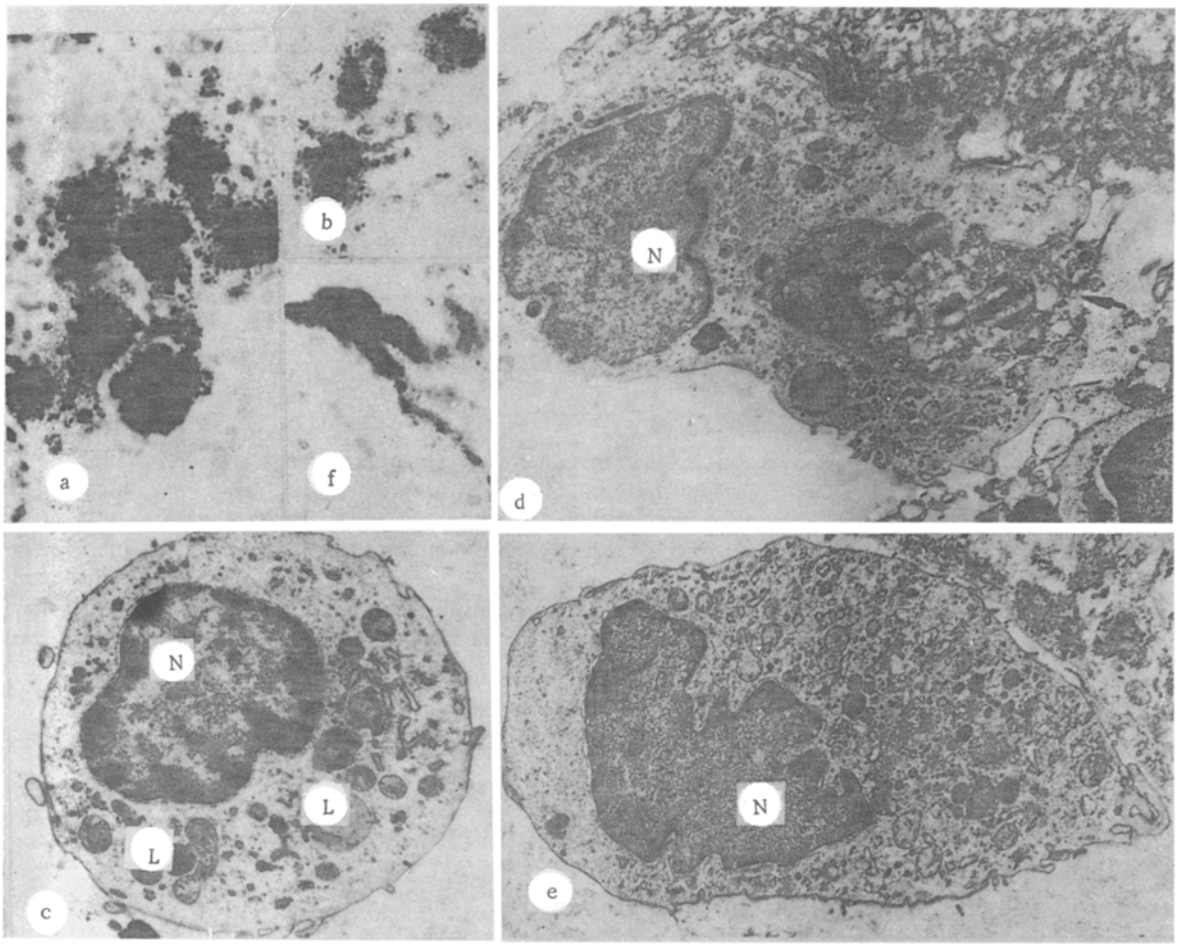

Fig. 1. Morphological changes in tissues of gunshot wound in rabbit (1st-3rd days): a) numerous granules with high AlP activity in polymorphs (1st day, experiment). Azo-coupling reaction, 1000 ×; b) few granules containing AlP in polymorphs (1st day, control). Azo-coupling reaction, 10,000 ×; c) macrophage with numerous lysosomes (L). N) Nucleus (1st day, experiment), 18,000 ×; d) actively phagocytic macrophage (3rd day, experiment). Arrow indicates tissue debris undergoing phagocytosis, 3300 ×; e) macrophage with signs of small-scale phagocytic activity (3rd day, control), 3300 ×; f) high AlP activity in fibroblast (3rd day, experiment). Azo-coupling reaction, 10,000×.

in the polymorphs of the experimental animals was higher on the first day (Fig. 1a, b), fell toward the 3rd day, and was lower after 5-7 days. Polymorphs forming the demarcation barrier, which was much narrower than in the control, were particularly active. After 1 day, single macrophages, round in shape and with an eccentric nucleus, cytopodia, and lyosomes were seen in the wound tissues of rabbits receiving cystamine (Fig. 1c). Similar cells appeared in the control, but not until the 3rd day (Fig. 1e). In the region of the subcutaneous muscle newly formed blood capillaries were found, with macrophages and randomly oriented fibroblasts, characterized by high AlP activity, between them (Fig. 1f). On the 3rd day numerous macrophages, actively phagocytosing cell debris and polymorphs (Fig. 1d), and distinguished by high AcP activity, appeared in the subcutaneous connective tissue and around the necrotic muscle fibers.

A fairly wide layer of granulation tissue, consisting of radially oriented blood vessels, amorphous ground substance, and circularly oriented fibroblasts [3], was formed in wound tissues of the experimental rabbits by the 5th day.

High AlP and diaphorase activity was found both in the wall of the blood vessels and in fibroblasts. Nearer the surface of the wound was an avascular layer consisting chiefly of separate fibroblasts. The wound surface was covered by a narrow mixed leukocytic and necrotic layer, beneath which were numerous macrophages and solitary leukocytes. The growing regen-

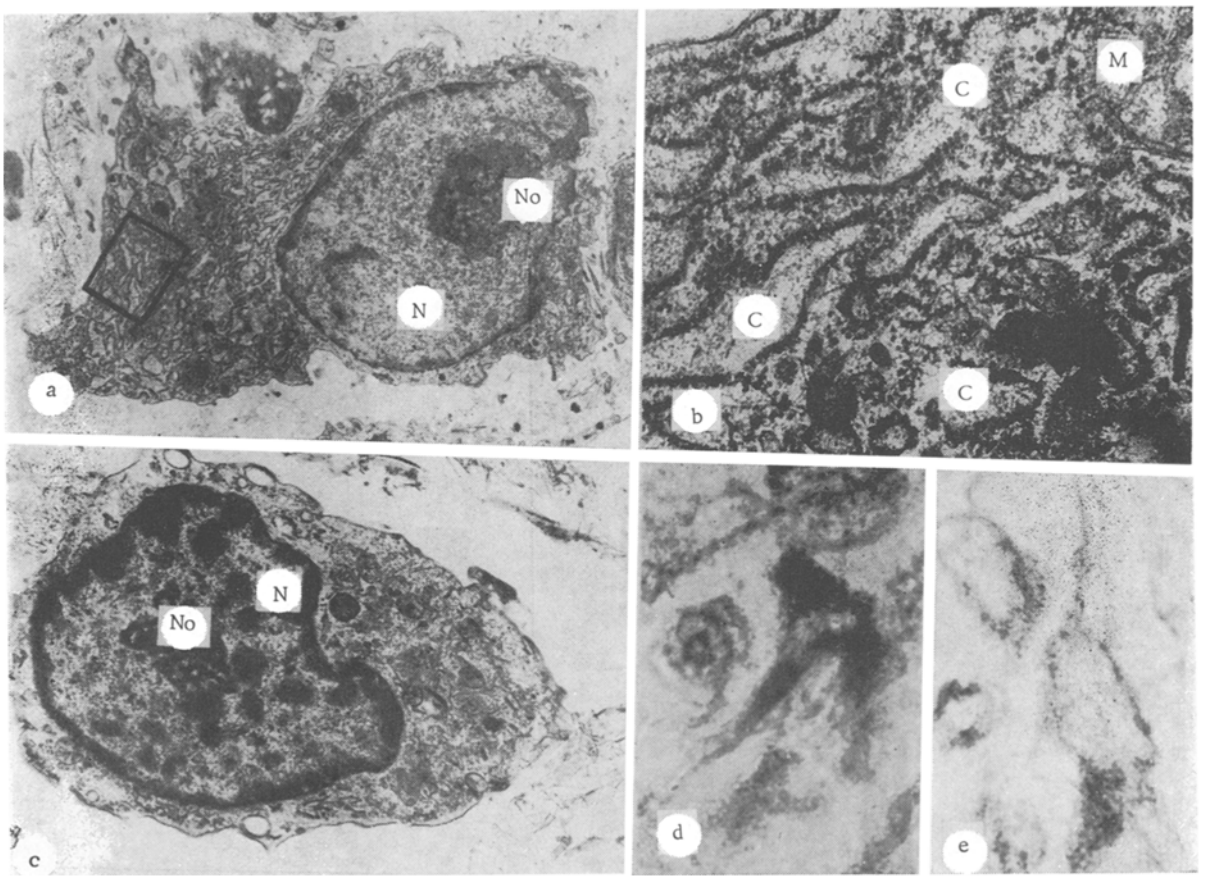

Fig. 2. Morphological changes in tissues of gunshot wound in rabbit (9th day): a) fibroblast with signs of high synthetic activity (experiment). No) Nucleolus. 5000 ×; b) cytoplasm of fibroblast (fragment of Fig. 2a). C) Cisterns of rough endoplasmic reticulum; M) mitochondria. 16,000 ×; c) immature fibroblast (control), 5000 ×; d) high AcP activity in fibroblast (control). Azo-coupling reaction, 1000 ×.

erating epidermal tissue covered a larger area of the wound than in the control. After 1 week the whole surface of the wound channel in rabbits receiving cystamine consisted of a continuous layer of granulation tissue, in which is layer of radially oriented blood vessels and of circularly oriented fibroblasts could be distinguished. Focal leukocytic infiltration continued around necrotic muscle fibers and in the surface-necrotic layer.

After 9 days the wound surface in the control animals was covered by a wide leukocytic-necrotic layer. Beneath this was a layer of amorphous granulation tissue, in which the fibroblasts showed initial signs of activation of synthesis (Fig. 2c). Meanwhile, in animals receiving cystamine, maturation of the granulation tissue in the gunshot wound was taking place much more actively. Nuclei of fibroblasts with dispersed chromatin contained large nucleoli; in the cytoplasm there were a branched network of cisterns of the rough endoplasmic reticulum filled with finely granular contents, a well developed lamellar complex, and microfilaments and mitochondria (Fig. 2a, b). In some cases active collagen formation by fibroblasts was combined with the formation of dense connective tissue in the depths of the wound. In the experimental rabbits, unlike in the controls, cells of the layer of circular fibroblasts gave a strongly positive reaction for AcP (Fig. 2d, e). In both groups of animals AlP and NADH-diaphorase were discovered in the endotheliocytes and periadventitial fibroblasts.

Three weeks after PST the wound surface in experimental animals receiving cystamine was completely epithelized. The newly formed epithelium had the appearance of a somewhat thickened sheet of tissue with well marked anisomorphism of its cell layers; inflammatory proliferation was almost completely absent in it. Mature fibroblasts and fibrocytes predominated in the granulation tissue (Fig. 3a, c). The cytoplasm of the fibrocytes was reduced over a considerable extent and consisted of chaotically oriented microfilaments, single short

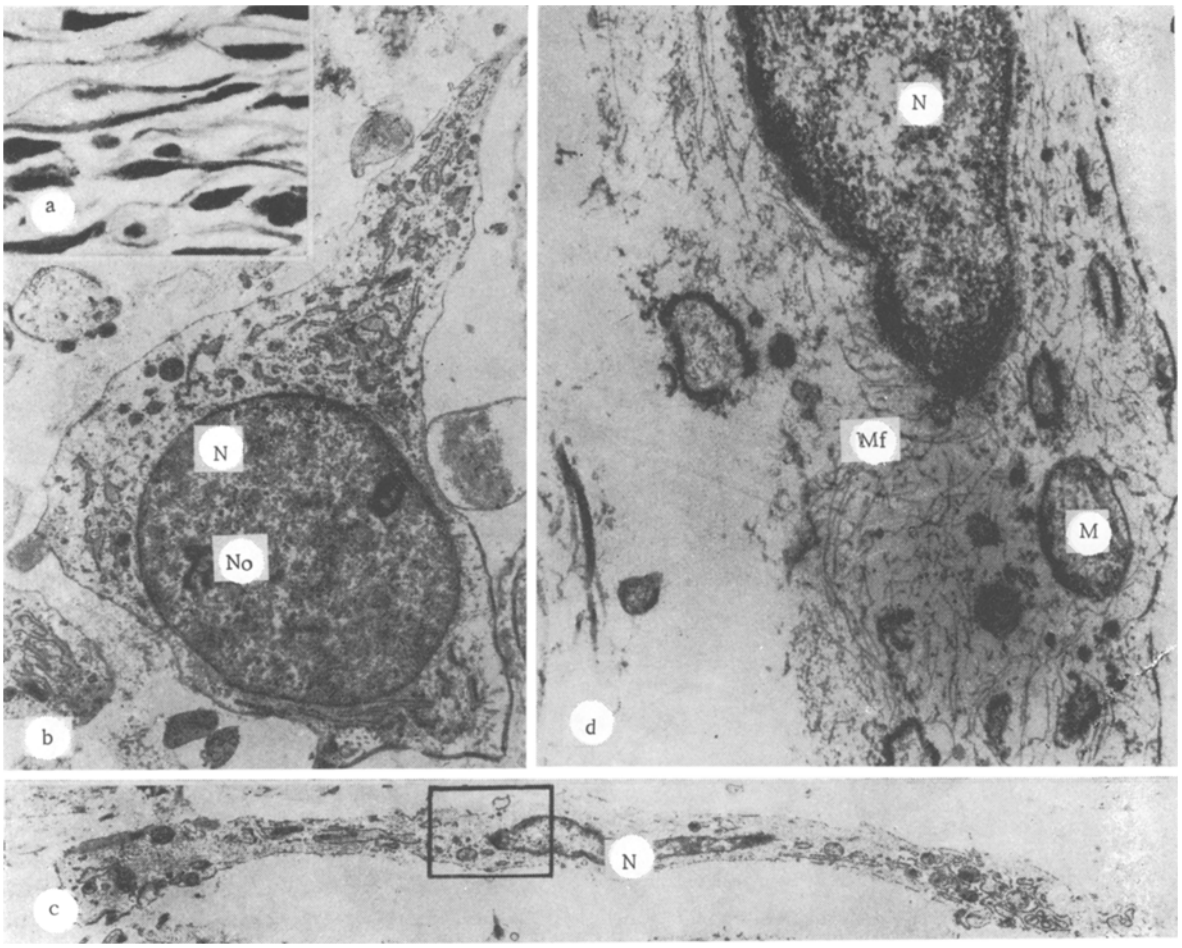

Fig. 3. Morphological changes in tissues of gunshot wound in a rabbit (28th day): a) mature granulation tissue (experiment). Semithin section. Toluidine blue, basic fuchsine, 400 ×; b) young fibroblast, 3300 ×; c) fibroblast (experiment), 2600 ×; d) fragment of Fig. 3c. N) Nucleus of fibrocyte; Mf) microfilaments, 16,000 ×.

cisterns of the rough endoplasmic reticulum, and a few ribosomes (Fig. 3d). In the control the wound surface was not epithelized until the 28th day, and macrophages and single polymorphs as well as young fibroblasts appeared in the granulation tissue (Fig. 3b).

Intravenous injection of cystamine into rabbits after a gunshot wound of the soft tissues of the thigh thus shortens the inflammatory reaction and promotes the earlier appearance of functionally active macrophages and of differentiated fibroblasts with high collagen-synthesizing activity. These processes accelerate maturation of granulation tissue and promote rapid epithelization of the wound surface. Under the influence of cystamine healing of gunshot wounds in rabbits takes place 4 or 5 days earlier than in the control.

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ACTIVATION OF CELL DIVISION AND NUCLEIC ACID SYNTHESIS IN THE CORNEAL EPITHELIUM OF ALBINO RATS BY REPEATED STRESS

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UDC 612.312.3+612.841.1./014.2:612.6/06:612.73.2

KEY WORDS: stress; nucleic acid synthesis; mitosis.

Adaptation to unfavorable factors is accompanied by activation of nucleic acid and protein synthesis in systems responsible for adaptation [5].

In this investigation the possibility of similar changes taking place in structures not actively participating in adaptation was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 112 male albino rats weighing 160-190 g. Stress was induced by exposure for 1.5 h to sublethal (up to 41.5°C) hyperthermia, exposure of animals in a pressure chamber to an "altitude" of 9000 m for 4 h, and fixation of the animals in the supine position for 1 h, all these procedures being applied once or five times. The techniques of hyperthermia and hypoxia were described previously in greater detail [1, 7]. The animals were killed and the corneas removed for investigation within 1 h after the end of exposure to stress. The rate of RNA synthesis was judged from the mean number of grains of silver above 100 labeled cells. The corneas were preincubated with [^{3}H]uridine (10 μ Ci/ml, specific radioactivity 1.5 mCi/ml, 28 Ci/mmole) for 1.5 h. The mitotic index (MI), the index of [^{3}H]thymidine-labeled nuclei (ILN), and the intensity of thymidine labeling (LI) were determined by methods described previously [8].

EXPERIMENTAL RESULTS

The results indicate that after a single exposure to hypoxia, hyperthermia, and immobilization, MI in the corneal epithelium decreased (Table 1). DNA synthesis under these circumstances remained stable. These data are in harmony with views on reactive inhibition of mitosis during stress [9, 10]. A reduction of 2.2 times in the intensity of thymidine labeling in the corneal epithelium after single sublethal hyperthermia was evidently the result of the direct action of the high temperature [12]. A single exposure to immobilization caused a significant decrease in the intensity of [^{3}H]uridine labeling, whereas hyperthermia led to no significant changes in this parameter.

A different picture was found after repeated exposure to stress. After immobilization five times reactive inhibition of mitosis was absent, whereas after hyperthermia and hypoxia MI increased by 1.7 times. Another significant difference from the results obtained with a single exposure to stress was activation of DNA synthesis. This was shown by an increase in ILN by 2.1 times after five exposures to immobilization and hyperthermia and by 1.7 times

Department of Pathological Physiology and Central Research Laboratory, Khabarovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 12, pp. 727-728, December, 1984. Original article submitted January 30, 1984.

0007-4888/84/9812-1717$08.50