RELATIVE VOLUME OF MEMBRANE MICROFLORA AND EPITHELIOCYTE MORPHOLOGY AFTER COMBINED HELIUM-NEON LASER IRRADIATION OF CHRONIC GASTRIC ULCERS AND VAGOTOMY

I. M. Baibekov, R. Sh. Mavlyan-Khodzhaev, and É. Sh. Musaev

UDC 616.33-002.44-089:616.833.191-089.85-059:615.849.19/-089.168:[616.33-018.7+616.33-008.87

KEY WORDS: gastric ulcers; microflora; helium-neon laser; vagotomy.

The relative volume of the microflora located directly on the surface of epitheliocytes in different parts of the stomach, especially the pyloroantral part, to which the name membrane microflora (MM) has been given, is increased in chronic ulcers and after vagotomy [4]. The gastric microflora and, in particular, *Campylobacter pyloridis*, are ascribed an important role in the development of gastritis and peptic ulcer [8, 12, 13].

It is also known that irradiation of the gastric mucosa (GM) by a helium-neon laser (HNL) has a stimulating effect on proliferative processes and induces changes in intracellular ultrastructures [3], indicating intensification of the specific function of the cells [1, 6, 10]. This is the reason for the clinical application of HNL to accelerate healing of gastroduodenal ulcers [7].

However, no investigations into the effect of the combined action of vagotomy and HNL on the relative volume of MM in the cells of GM have yet been undertaken. The investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

The effect of HNL irradiation of chronic experimental gastric ulcers on the relative volume of MM and of epitheliocytes of GM was studied. Experimental ulcers, 33 days after their induction by Okabe's method [2] were irradiated by HNL through a light guide introduced into the cavity of the stomach. Irradiation was carried out 3 times at intervals of 1 day. The optimal sessional dose of each irradiation was established by the writers previously and shown to be 20 J/cm^2 for a duration of 3 min [5]. The light guide introduced into the cavity of the stomach was fixed strictly above the region of the ulcer, at an equal distance above its surface, as was confirmed by visual control [9]. Gastric ulcers of rats undergoing subdiaphragmatic vagotomy 30 days after induction of the ulcer, also were irradiated by HNL. Vagotomy also was performed on animals without ulcers. The rats were killed by instant decapitation 30, 40, 60, and 90 days after induction of the gastric ulcer and 10, 30, and 60 days after vagotomy. Animals irradiated by HNL were killed not earlier than 3 days after the last session, 40-45 days after induction of the gastric ulcer (Table 1). Intact animals and animals irradiated with white light through a light guide introduced into the cavity of the stomach served as the controls. Altogether 77 Wistar rats weighing not less than 140 g were used. All manipulations were carried out under ether anesthesia. Excised pieces of the stomach wall with ulcers and outside the ulcer region were treated by the ordinary methods for light and electron microscopy; semithin sections were stained with methylene blue and fuchsine. The relative volume of MM was determined at a distance of up to 40 μ from the surface of the epitheliocytes in the region of the ulcer and the fundal and pyloroantral portions under a magnification of 900 ×, whereas the relative volume of the cells of the fundal glands was determined under a magnification of 400 times by stereometry on semithin sections.

Laboratory of Pathological Anatomy, Tashkent Branch, All-Union Scientific Center for Surgery, Academy of Medical Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 1, pp. 106-108, January, 1989. Original article submitted February 20, 1988.

 0007-4888/89/0001-0126$12.50

TABLE 1. Relative Volume of MM after Vagotomy (V) and Irradiation of Gastric Ulcer (GU) by HNL

Procedure and time after it, days	Fundal division	Region of ulcer	Pyloric division
1. Control	5,1±0,6	—	9,6±0,2
2. GU 30	14,7±0,2	—	20,2±0,8
3. GU 40	8,3±0,5	22,6±1,2	20,2±0,8
4. GU 60	9,1±0,1	—	18,1±0,4
5. GU 90	4,7±0,1*	—	7,1±0,5
6. V 10	12,7±0,4	—	20,7±1,4
7. V 30	14,9±0,6	—	26,6±3,3
8. V 60	5,7±0,1*	—	19,6±0,1
9. GU 40, V 10	10,7±1,0**	30,2±1,4	25,3±1,7
10. GU 60, V 30	—	—	34,3±0,2
11. GU 90, V 60	8,9±0,5	—	17,2±0,3
12. GU 40, HNL	4,8±0,4	11,7±0,7	11,0±0,7
13. GU 40, V 10 HNL	5,6±0,5*	3,1±0,5	—

Legend. Significance of difference (p) calculated during comparison of Nos. 2-8 with No. 1, of Nos. 9-11 with Nos. 3-5, No. 12 with No. 3, and No. 13 with No. 9: *p>0.1, **p<0.05, in all other cases p< 0.01.

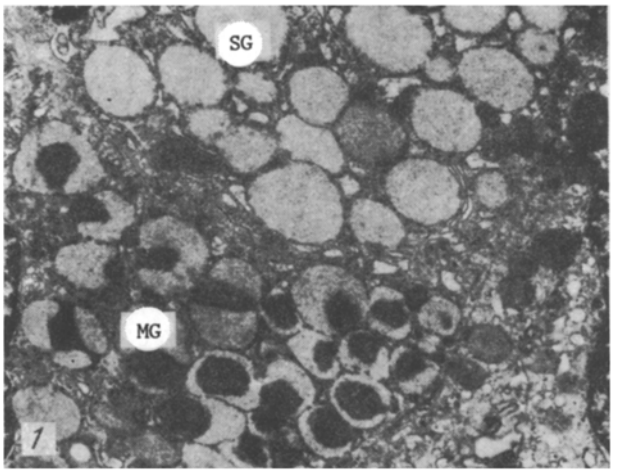

Fig. 1

Fig. 2

Fig. 1. Chief cells with typical secretory granules (SG) and mixed granules (MG). Forty days after induction of gastric ulcer and 10 days after vagotomy. 10,000 ×.

Fig. 2. Accumulation of microorganisms on surface of epithelial pit cells at border of ulcer, with variation in content of mucoid. Forty days after induction of gastric ulcer. Methylene blue — fuchsine. 400 ×.

EXPERIMENTAL RESULTS

Induction of chronic acetate ulcers caused an increase in the relative volume of the chief cells from 30.8 ± 1.2% in the control to 37.3 ± 0.5% ($p<0.01$) and of the parietal cells from 34.4 ± 1.5 to 40.3 ± 0.6% ($p<0.01$) 40 days after induction of the gastric ulcer. At this time the relative volume of the mucocytes fell from 24.8 ± 1.3 to 11.0 ± 0.4% ($p<0.01$) correspondingly. A similar tendency also was observed at other times. Vagotomy led to a decrease in the relative volume of both chief and parietal cells. For instance, 60 days after vagotomy the relative volume of the chief cells was 25.6 ± 1.4% ($p<0.01$), of the parietal cells 29.1 ± 2.1% ($p<0.05$) and of the mucocytes up to 31.7 ± 1.8% ($p<0.01$). Vagotomy carried out for gastric ulcer also caused a decrease in the relative volume of the parietal and chief cells to values characteristic of the control animals. The state of their intracellular structures was evidence of reduced ability of the cells to perform their specific secretory function.

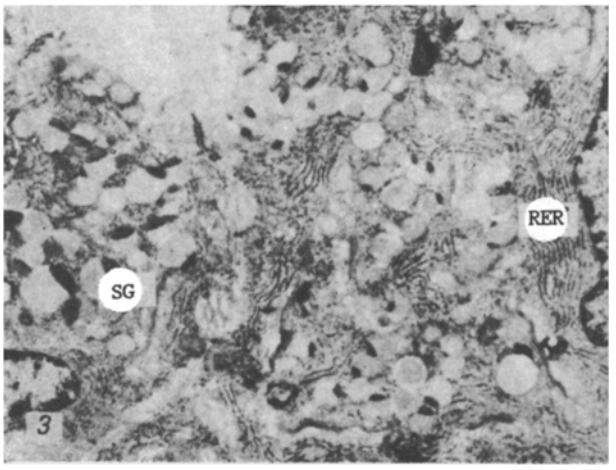

Fig. 3

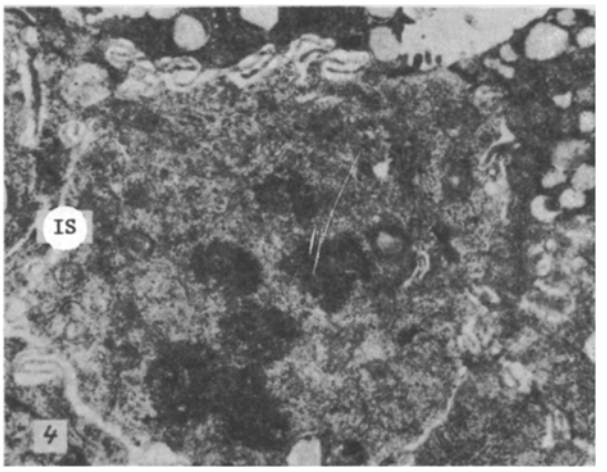

Fig. 4

Fig. 3. Mucocytes with polymorphic secretory granules (SG) and with well-developed rough endoplasmic reticulum (RER).

Fig. 4. Mitosis of gland neck cell, dilated intercellular spaces (IS). Forty-five days after induction of gastric ulcer with irradiation by HNL together with vagotomy. 10,000 ×.

The extent of the membranes, tubulovesicles, and intracellular secretory channels and the volume of the mitochondria were reduced in the parietal cells, but the number of free polysomes was increased. The areas of cross section of the rough endoplasmic reticulum and Golgi complex were reduced in a high proportion of chief cells. So-called mixed granules appeared (Fig. 1), evidence of the "mucoidization" of the chief cells [14]; all this points to a disturbance of "differentiation" of the chief and parietal cells of the fundal glands, and of changes in them reflecting a disturbance of regeneration [11].

Gastric ulcers cause a marked increase in the relative volume of MM, especially in the early stages, to reach the control level at the later times of observation in both fundal and pylorantral divisions. Vagotomy, performed both on intact animals and on animals with induced gastric ulcers, also caused an increase in the relative volume of MM (Table 1, Fig. 2). Following irradiation of the gastric ulcer by HNL, accompanied or not by vagotomy, led to a significant decrease in the relative volume of MM in all parts of the stomach investigated, but more particularly in the fundal division, where it actually fell below the control level.

The increase in the relative volume of MM was accompanied by polymorphism of the cells and disturbance of the integrity of their apical parts; marked unevenness and a general decrease in the mucoid content was observed in the pit surface mucocytes (Fig. 2).

Irradiation of the ulcers with HNL caused both a decrease in volume of MM and changes in the intracellular structures of the chief, parietal and, in particular, the mucoid cells, evidence of their more rapid differentiation. The extent of the rough endoplasmic reticulum in the mucocytes was increased, as also was the number of secretory granules (Fig. 3). The proliferative activity of the cells at the edge of the ulcer was increased, as was confirmed by electron microscopy (Fig. 4).

The investigation thus showed that irradiation of gastric ulcers by HNL coupled with vagotomy causes reduction of regeneration-disturbing changes in the mucocytes and other cells of the fundal glands and reduces the relative volume of the membrane microflora.

LITERATURE CITED

1. A. P. Avtsyn and V. A. Kakhlamov, Ultrastructural Principles of Cell Pathology [in Russian], Moscow (1979).
2. L. I. Aruin, B. Z. Chikunova, and I. E. Trubitsina, Current Problems in Gastroenterology [in Russian], Vol. 2, No. 9, Moscow (1976), pp. 97-109.
3. I. M. Baibekov and É. Sh. Musaev, Byull. Éksp. Biol. Med., No. 10, 501 (1981).
4. I. M. Baibekov and R. Sh. Mavlyan-Khodzhaev, Byull. Éksp. Biol. Med., No. 4, 499 (1987).
5. I. M. Baibekov and É. Sh. Musaev, Abstracts of Proceedings of the 4th All-Union Conference on Cell Pathology [in Russian], Moscow (1987), p. 108.

6. K. A. Zufarov, Structural Principles of Compensatory-Adaptive Processes [in Russian], Tashkent (1976).
7. V. N. Koshelev, Laser Therapy of Gastroduodenal Ulcers [in Russian], Saratov (1986).
8. A. S. Loginov, L. I. Aruin, and I. A. Smotrova, Klin. Med., No. 8, 20 (1987).
9. É. Sh. Musaev, Dokl. Akad. Nauk Ukr. SSSR, No. 8, 55 (1986).
10. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, Adaptive Restructuring of Biorhythms [in Russian], Moscow (1975).
11. Structural Bases of Adaptation and Compensation of Disturbed Functions [in Russian], ed. by D. S. Sarkisov, Moscow (1987).
12. C. A. M. McNulty and R. Wise, Br. Med. J., 291, No. 6492, 367 (1985).
13. B. J. Rathbone, J. I. Wyatt, and R. V. Heatley, Gut, 27, No. 6, 635 (1986).
14. S. Susuki, S. Tsuyama, and F. Murata, Cell Tissue Res., No. 3, 475 (1983).

CULTURE OF EPIDERMOCYTES ON A COLLAGEN SUBSTRATE

A. A. Pal'tsyn, G. G. Serov,
R. P. Gasanov, and V. P. Tumanov

UDC 616.5-001.17-089.843

KEY WORDS: epidermocytes; tissue culture; collagen substrates.

A new trend has recently been formed in the treatment of burns, involving attempts to grow in culture, from a small piece of skin taken from a patient, much larger sheets of cells suitable for transplantation [1, 2, 4]. The development of techniques for transferring such an epidermal sheet to the wound surface is of great importance [1-3, 6]. To preserve the proliferative ability of the basal cells of the epithelium and to prevent retraction of the epidermal sheet thus obtained after its detachment from the floor of the culture vessel, various biological and synthetic substrates have been suggested (collagen lattice [1], stage I and stage II [2]).

In the investigation described below, the proliferative activity and rate of formation of the epidermal sheet during culture of epidermocytes on a collagen substrate were studied by modern methods.

EXPERIMENTAL METHOD

Pieces of skin obtained from patients during skin autografting and also split skin grafts obtained from cadavers served as the test material. Skin grafts 0.2-0.3 mm thick were taken with a DRM-60 dermatome and placed in a test tube containing 25 ml of Eagle's nutrient medium with antibiotics for 2 to 20 h. The nutrient medium with a high concentration of antibiotics was changed for fresh solution of the same composition, but containing only 1-20th of the concentration of antibodies. This solution was then poured off and the skin graft rinsed for 20 min in 0.02% EDTA and kept for 18-20 h in a 0.25% solution of trypsin at 4°C. After this stage the trypsin solution was poured off and the skin graft rinsed with Na-phosphate buffer, pH 7.2-7.4, and transferred to a Petri dish with medium containing calf serum to neutralize the enzyme, where the epidermis was separated from the dermis. The epidermocytes were isolated by repeated pipeting. After trypsinization by the method described above, the number of epidermocytes isolated from 1 cm^2 of skin graft was 10^5-10^6 cells. On supravital staining with a 1% solution of trypan blue, viable cells accounted for 90-95% of the total number of cells in the suspension, depending on the state of the initial skin. The cell suspension was harvested in a test tube and the cells were sedimented by centrifugation at 500 rpm for 10 min. The supernatant was poured off, then resuspended in hypocalcium medium (Ca^{++} concentration 0.14 mM) containing glutamine and Ultroser. The number of cells was counted in a Goryaev's

Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 1, pp. 109-111, January, 1989. Original article submitted April 29, 1988.

