LITERATURE CITED

1. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Damage [in Russian], Moscow (1984).
2. V. V. Khlystov and V. S. Pavlenko, Cytomedins. Their Function in the Body and Use in Clinical Practice [in Russian], Tomsk (1986), pp. 59-62.
3. V. V. Khlystov, V. S. Pavlenko, V. Kh. Khavinson, et al., Arkh. Patol., No. 9, 27 (1989).
4. A. Cryer and W. Bartley, Biochem. J., **134**, No. 4, 1119 (1973).
5. R. B. Jennings and K. A. Reimer, Am. J. Pathol., **102**, 241 (1981).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., **193**, No. 2, 265 (1951).
7. U. F. Rassmussen, FEBS Lett., **19**, No. 3, 239 (1971).
8. W. Rouslin and R. W. Millard, Am. J. Physiol., **240**, H308 (1981).
9. A. Tragologg, Mitochondria, New York (1982), p. 342.
10. G. V. Vahouny, R. Wei, R. Starkweather, and C. Davis, Science, **167**, 1616 (1970).

USE OF THE NEW MUSCARINIC CHOLINOLYTIC KG-62 TO CORRECT THE COURSE OF EXPERIMENTAL GASTRIC ULCER

A. B. Denisov, L. S. Kulikov, N. A. Kardanov, N. N. Godovikov, R. A. Druzhinina, and E. N. Pomerantseva

UDC 616.33-002.44-085.217.34-036.8-092.9-07

KEY WORDS: muscarinic cholinolytic KG-62; gastric ulcer in rats.

Muscarinic cholinolytics, which inhibit the acid-forming function of the stomach and restore its normal motor activity, are widely used in therapeutic schedules aimed at correcting gastric function in patients with peptic ulcer [5]. One widely used drug is metacin (oxyphenonium bromide), which, because of its chemical structure, has an exclusively peripheral muscarinolytic action, thereby ensuring absence of many side effects A series of iodomethylates of oxyalkylphosphinic acids with muscarinolytic action has been synthesized at the N. A Nesmeyanov Institute of Organoelementary Compounds, Academy of Sciences of the USSR [2]. One of the most active of these compounds has proved to be the substance conventionally named KG-62. Our investigations on animals showed that KG-62 in fact possesses a muscarinic cholinolytic action similar to that of metacin [3, 4].

The aim of this investigation was to assess the efficacy of KG-62 on the course of experimental gastric ulcer.

EXPERIMENTAL METHOD

Experiments were carried out on 46 noninbred rats weighing 160-200 g, anesthetized with hexobarbital (70 mg/kg). An experimental ulcer was produced with glacial acetic acid [11], for this model most completely reproduces the morpho-

Department of Pathophysiology, Faculty of Stomatology, N. A. Semashko Moscow Medical Stomatologic Institute. Laboratory of Biologically Active Organophosphorus Compounds, A. N. Nesmeyanov Institute of Organoelementary Compounds, Academy of 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. 112, No. 7, pp. 27-30, July, 1991. Original article submitted July 5, 1990.

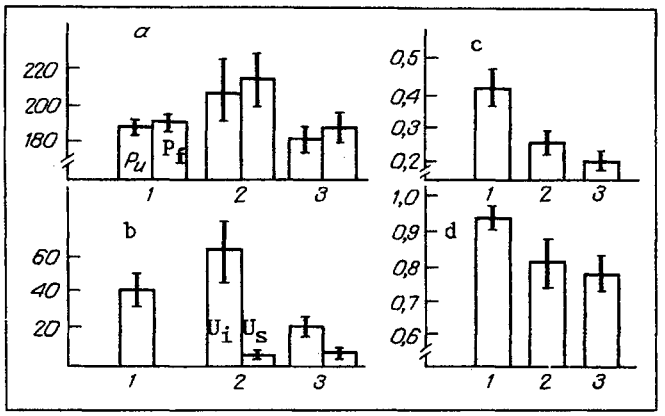

Fig 1. Changes in biometric parameters on 7th day of treatment of acetate ulcer by muscarinic cholinolytics. 1) Groups of animals receiving physiological saline, 2) metacin, 3) KG-62. a: Ordinate, weight of rats (in g), P_i) initial weight of rats, P_f) final weight of rats. b: Ordinate, area of ulcers (in mm^2), U_l) large ulcers, U_s) small ulcers; c: ordinate, thymic index (conventional units), d: ordinate, splenic index (conventional units).

logical picture of the ulcer in man [6]. Animals with acetate ulcer were divided into three groups: rats of group 1 received physiological saline (control), those of group 2 received metacin, and those of group 3 received KG-62 The drugs were dissolved in physiological saline and injected intramuscularly twice a day in a dose of 5 mg/kg and in a volume of 0.5 ml. The animals were killed on the 7th and 14th days. The effect of muscarinic cholinolytics on the course of the ulcer was studied on the basis of morphometric, histologic, and biochemical parameters. As the stress control, we estimated the weight of the thymus and spleen, and calculated the thymic and splenic indices. The thymus and spleen were removed and dried at 110°C for 24 h. After removal of the stomach, the area of the ulcer was measured and pieces of tissue from the region of the ulcer and the intact stomach wall were studied biochemically and histologically. In order to study enzyme activity in the tissue, activity of lactate dehydrogenase (LDH) [12], alkaline phosphatase (AlP), and acid phosphatase (AcP) were determined at pH 4.8 [7]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

In acetate ulcer, no significant changes took place in the mean weight of the rats on the 7th day of the experiment, but substantial atrophy of the thymus and spleen was found in rats receiving KG-62 (Fig 1). Later (14th day) the weight of these lymphoid organs did not differ from the control.

At autopsy on the animals gastric ulcers were found, in most cases with penetration. In animals receiving muscarinic cholinolytics, no abnormallties were found except in rats receiving KG-62: in these animals the number of adhesions between the stomach and surrounding tissues was appreciably smaller and they were more delicate.

It will be clear from Fig. 1 that the method of producing an acetate ulcer which we used caused the development of an ulcer with a mean area of 42 ± 12 mm^2 by the 7th day, If the area of the ulcer in rats receiving physiological saline is taken as 100%, in rats receiving metacin the mean area of the ulcer was 69%, whereas under the influence of KG-62, it was only 27% of the control level. Statistical analysis revealed great scatter of the data. The coefficient of variation in group 2 was 136% and in group 3 it was 99%. Statistical analysis of these results showed significantly the existence of two independent samples. In Table 1, two separate columns are accordingly used for the experimental groups: "large" ulcers and "small" ulcers. This subdivision was made conventionally on the basis of variance analysis of the areas of the ulcers in the group. In group 2 the mean area of the ulcer in 44% of rats was actually greater than in the control, and in the remaining rats, the ulcers had almost healed. In group 3 the area of the large ulcers was only half of that in the control (44%). In other animals the area of the ulcers was 10% of the control, Thus in the early stages of the experiment the substances used

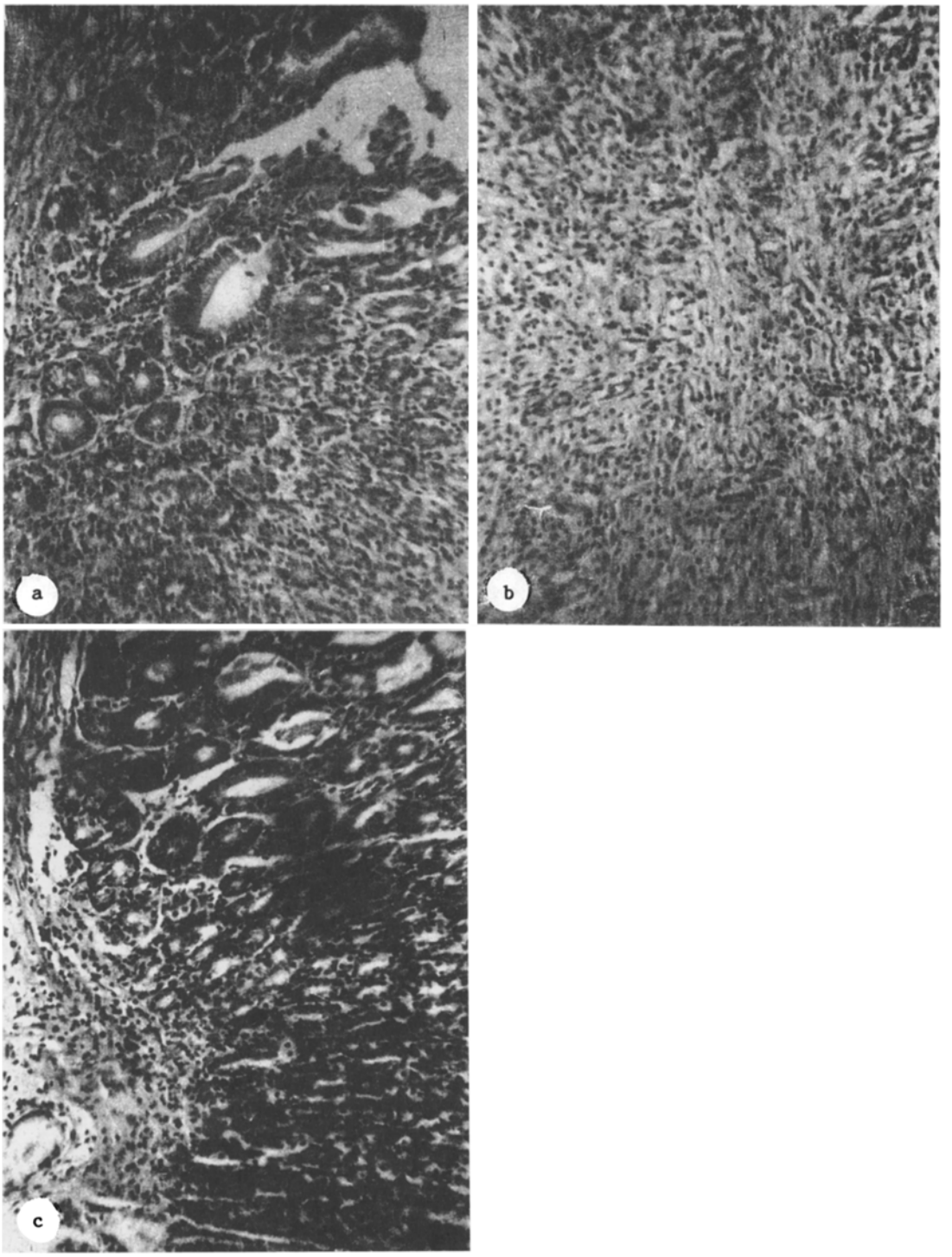

Fig 2. Morphology of gastric wall during healing of experimental acetate ulcer after injections of KG-62 and metacin. a) 7th day of healing of ulcer after treatment with KG-62. Floor of ulcer covered with newly formed mucosa. Hematoxylin-eosin, 70×; b) 7th day of experiment with injections of KG-62. Floor of ulcer represented by thick collagen fibers. Hematoxylin-eosin, 70×; c) 14th day of ulcer development after treatment with metacin. Formation of mucosa in region of floor of ulcer. Hematoxylin-eosin. 70×.

reduced the area of the experimental ulcer, but KG-62 was found to be more effective than metacin. Later, on the 14th day, the ulcers in most rats had almost completely healed.

TABLE 1. Specific Activity of Gastric Wall Enzymes of Rats with Acetate Ulcer (in μmoles substrate/min/g protein) after Treatment with Muscarinic Cholinolytics for 14 Days (M ± m)

Time of investigation	Enxyme	Physiological saline (co		Metacin		KG-62	
		I	II	I	II	I	II
7th day	AcP	6,1±1,0	65±8	54±12	69±4	58±11	67±6
	AlP	71±5	90±9	82±9	91±12	70±10	102±9
	LDH	312±47	153±16	243±86	202±26	402±38	136±11
14th day	AcP	73±22	135±34	44±7	211±10	71±22	219±37
	AlP	86±21	57±10	71±16	165±18	90±16	132±26
	LDH	336±46	339±78	349±80	457±43	384±54	302±34

Legend. I) Intact stomach tissue, II) region of ulcer.

The results of the microscopic and biometric investigations confirmed the microscopic findings. Compared with the control group, on the 7th day epithelization was more intensive and extensive. During proliferation of glandular structures there was a tendency toward differentiation of the epithelial cells of the mucous membrane, as shown by the appearance of mucous glands at these times (Fig. 2a). Collagen formation took place more intensively in the submucosa and, in particular, in the muscular layer, a finding confirmed by the marked proliferation of fibroblasts associated with definite activity of endothelial cells in the newly formed blood vessels (Fig. 2b). Components of fibrinoid necrosis and perifocal inflammation were considerably depressed at this time already.

Comparison of the control and experimental material revealed complete epithelization of the ulcer surface with the formation of mucous membrane of pyloric type in the animals receiving muscarinic cholinolytics (Fig. 2c). The completed processes of regeneration of the mucous membrane with differentiation of epithelial and gland cells were accompanied by the complete formation of connective tissue also in the submucosa and muscular layer, accompanied by a decrease in the number of fibroblasts, although vascularization still remained quite intensive. The formation of new vessels with a large capillary network in the floor of the healing ulcer ensured the high level of nutrition of the injured stomach wall. It can be tentatively suggested that the use of muscarinic cholinolytics not only promotes adequate repair of connective tissue and epithelium with elements of structural and functional differentiation, but also maintains the level of cell proliferation without any sign of excessive hyperplasia of the mucous membrane and connective scar tissue.

Biochemical investigations of the gastric tissue revealed (Table 1) reduction of LDH activity by half in the animals in the region of the ulcer. Under the influence of metacin, LDH activity in the intact tissue was reduced, but in the region of the ulcer the opposite response was observed Injection of KG-62 increased LDH activity in the intact tissue by 29%, without any effect on this parameter in the region of the ulcer.

On the 14th day, in rats of all groups, activity of AcP in the region of the ulcer was more than twice its activity in intact tissue ($p < 0.01$). AcP activity also was higher than on the 7th day of the experiment. In rats receiving metacin, both AlP activity (by 132%, $p < 0.01$) and LDH activity (by 31%, $p < 0.05$) were significantly increased in the region of the ulcer. In animals receiving KG-62, AlP activity was increased, but not significantly, and LDH activity was reduced (by 31%).

Thus whereas metacin had no significant effect on glycolysis in the tissues of the stomach, KG-62 depressed LDH activity in the region of the ulcer and stimulated it in in act tissues (on the 7th day). Since pyruvic acid was used as substrate for determination of LDH, it can be postulated that the preparation KG-62 activates anaerobic glycolysis in intact tissue but inhibits these processes in the region of the ulcer. Since potentiation of glycolysis is characteristic of actively regenerating tissues, it can be tentatively suggested that this fact is evidence of compensatory activation of physiological regeneration. Glycolysis is inhibited in the region of the ulcer, indicating protective inhibition of metabolism in the region of the pathological process. Metabolic changes were more marked after injection of KG-62.

The experiments thus showed that KG-62, which possesses a cholinolytic action, evidently closely resembles in its pharmacologic properties not only oxyphenonium bromiae, but also another selective muscarinic cholinolytic – pirenzepine (Gastrozepin) [3, 4, 8, 9]. It has a stronger action than oxyphenonium bromide, accelerating healing of experimental gastric ulcers. This effect is based not only on its inhibitory action on the secretion of hydrochloric acid and pepsinogen, but also, evidently, on activation of physiological regulation in intact tissue.

LITERATURE CITED

1. L. I. Aruin, Klin. Med., No. 2, 55 (1981).
2. R. I. Volkova et al., Dokl. Akad. Nauk SSSR, **262**, No. 1, 479 (1982).
3. A. I. Volozhin et al., Diagnosis and Treatment of Inflammatory and Degenerative Diseases of the Maxillofacial Region [in Russian], Smolensk (1988), pp. 64-67.
4. A. B. Denisov et al., Abstracts of Proceedings of the 4th All-Union Congress of Pathophysiologists [in Russian], Vol. 2, Kishinev (1989), p. 439.
5. A. Kh. Vasilenko (ed.), Handbook of Gastroenterology [in Russian], Moscow (1976).
6. B. B. Timashkevich and I. G. Zaporozhenko, Byull. Éksp. Biol. Med., No. 2, 226 (1980).
7. O. T. Bessey et al., J. Biol. Chem., **164**, 321 (1946).
8. R. Hammer et al., Nature, **283**, 90 (1980).
9. M. Leitold et al., Therapiewoche, **27**, 1532 (1977).
10. O. H. Lowry et al., J. Biol. Chem., **193**, 265 (1951).
11. S. Okabe et al., Am. J. Dig. Dis., **16**, No. 3, 277 (1971).
12. T. Wroblewski et al., Proc. Soc. Exp. Biol. (New York), **90**, 210 (1955).

ENERGY-DEPENDENT REGULATION OF LIVER CELL TRANSMEMBRANE POTENTIALS IN HYPOXIA

L. D. Luk'yanova, A. T. Ugolev, P. I. Fedorov, N. V. Lukinykh, and A. M. Dudchenko

UDC 612.015.3+612.017.4

KEY WORDS: hepatocytes; perfused liver; membrane potential; glycolysis; hypoxia

The development of pathological processes leading to irreversible destructive changes in the cell is accompanied by considerable changes in the level of the plasma membrane potential and, conversely, maintenance of that potential within certain limits is one of the most important conditions determining preservation of cell function. In precisely the same way, the mitochondrial membrane potential is an indicator of its integrity and functional normality of energy accumulation and transformation in the mitochondria. However, it is not yet clear what regulatory mechanisms control the stability of intracellular transmembrane potentials or whether functional correlation exists between them under normal and pathological conditions.

The solving of problems of this kind has become possible with the appearance in recent times of methods of recording plasma and mitochondrial membrane potentials in intact cells [1-4].

The investigation described below showed that formation and maintenance of mitochondrial and plasma membrane potentials of liver cells are interconnected and depend on energy pools of both mitochondria and cytosol.

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 7, pp. 30-32, July, 1991. Original article submitted November 29, 1990.

0007-4888/91/0007-0933$12.50