NETWORK COPOLYMERS OF ACRYLIC AND METHACRYLIC ACIDS: HYDROPHILIC OINTMENT BASES FOR WOUND-HEALING PREPARATIONS

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Investigations aimed at the development of a combined wound-healing preparation on a hydrophilic ointment base have been carried out. Copolymers of acrylic acid and its derivatives with a new divinyl monomer, β-vinyloxyethylamide of acrylic acid, are proposed as the bases. It is established that these ointment bases exhibit sufficiently high osmotic activity. Polymer-based complexes including the ethereal oil of smooth wormwood and the proteolytic enzyme protosubtilin showed pronounced antibacterial and wound-healing activity.

Despite the large number of preparations for the local treatment of purulent wounds and trophic ulcers, the desired wound-healing effect is far from being reached [1, 2]. Certain hopes are still related to the class of hydrocolloidal compositions proposed in the 1990s. Hydrocolloids represent polymers capable of swelling and dissolving in aqueous media with the formation of colloidal solutions. This class includes natural polysaccharides of plant, animal, and bacterial origin and some synthetic polymers. Hydrocolloids constitute a basis for some modern wound-healing compositions such as Granuloflex, Varihesive, Surgihesive, and DuoDerm [3 – 7]. Such hydrocolloid preparations effectively protect damaged tissues from overdrying and traumas, favor the lysis of necrotic tissues, and produce wound drainage. Another important property of hydrocolloids is their ability to stimulate reparative processes [8].

However, experience gained in the clinical use of hydrocolloidal wound-healing preparations revealed certain disadvantages. Preparations of the DuoDerm type (most widely used in many countries) have proved poorly effective in the treatment of deep wounds, burns, decubital ulcers with purulent manifestations, and trophic ulcers in patients with diabetes mellitus. Apparently, the efficacy of hydrocolloids decreases in cases of pathology involving inflammation because these compositions are deprived of antimicrobial properties [9]. In this context, attempts at modifying hydrocolloids or creating new related compositions for increasing the would-healing efficacy have been undertaken in recent years [10].

The local treatment of purulent wounds is traditionally carried out taking into account the phase of a particular process. However, large and deep wounds frequently feature different phases of wound development simultaneously. This circumstance suggests the need for developing universal preparations capable of combining the sorption effect, antimicrobial properties, and the ability to stimulate reparative processes.

Network (co)polymers of acrylic and methacrylic acids possess all the typical properties of hydrocolloids. Network polymers capable of forming hydrocolloids are usually obtained by means of chemical, thermal, or radiation-induced cross-linking. We have used chemical cross-linking for obtaining network copolymers of β-vinyloxyethylamide of acrylic acid (β-VEAA), whose structure contains two double bonds having significantly different activities and spaced at large distance from each other. Using these bonds, it is possible to control the degree of cross-linking in the course of copolymer synthesis and obtain gels possessing the desired sorption characteristics, structure, and mechanical properties [11]. We have developed a series of polymeric bases for hydrocolloidal compositions, representing copolymers of β-VEAA with acrylic acid (AA) and methacrylic acid (MAA) and terpolymers of β-VEAA with monoethanolamine monovinyl ether (MME) and the sodium salt of methacrylic acid (NaMAA).

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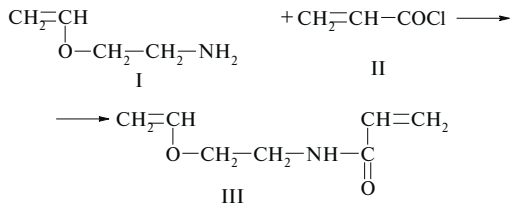
Using these polymeric bases, we created compositions where the role of active agents possessing anti-inflammatory, antimicrobial, and reparative properties is played by the ethereal oil of smooth wormwood (*Artemisia glabella* Kar. et Kir.) and the proteolytic enzyme protosubtilin known to be the parent substance in preparations such as immozimase and profezim [14].

The aim of this study was to evaluate the wound-healing properties of the proposed new polymer-based compositions.

EXPERIMENTAL CHEMICAL PART

The initial β-VEAA was synthesized via condensation of MME with AA chloroanhydride [11] according to the following scheme:



(yield, 82.5%; b.p., 90°C/2 Torr; d_4^{20}, 1.082; n_D^{20}, 1.4786).

The target product (III) was identified using the results of elemental analyses and the IR spectroscopic data.

The co- and terpolymers of β-VEAA were prepared using the joint polymerization of monomers (taken in various ratios) in aqueous ethanol at 60°C in the presence of AIBN.

The degree of monomer conversion in these syntheses amounted to 60 – 70 wt.%. The gels were washed from unreacted monomers for seven days with distilled water. The osmotic properties of gels were determined as described in [15].

The ethereal oil of smooth wormwood (EOSW) was a commercial preparation available from the Karaganda Pharmaceutical Plant (Kazakhstan), which met all requirements of the corresponding temporal pharmaceutical article (Kazakhstan Republic, VFS 42-1349-04). The proteolytic enzyme complex (PEC) of *Bacillus subtilis* NI-1 (containing alkaline and acid proteases) was obtained from the Institute of Pharmaceutical Biotechnology (Kazakhstan).

Experimental ointment based on β-VEAA-MAA copolymer. To a solution of the PEC powder (4.12 g) in 80 ml of phosphate buffer (pH 8.2) was added 4.12 g of the copolymer and the mixture was allowed to stand until gel formation. To this gel was added a solution of 0.83 g of surfactant (Tween-80) in 8.25 g of EOSW and then distilled water to a total weight of 100 g.

Experimental ointment based on β-VEAA–NaMAA–MME terpolymer. To a mixture of 5.12 g of the comminuted terpolymer and 2.56 g of PEC powder was added 80 ml of phosphate buffer (pH 8.2) and the mixture was allowed to stand until gel formation. To this gel were sequentially added 0.04 g of surfactant (Tween-80), 4.26 g of EOSW, and distilled water to a total weight of 100 g.

EXPERIMENTAL BIOLOGICAL PART

Wound-healing properties of EOSW–PEC ointments on the proposed gel base were studied in a group of white mongrel rats weighing 250 – 300 g with model purulent wounds. The wounds, of oval shape with lateral dimensions 3.5 × 2 cm, were formed on the back, where the skin (deprived of hair) and subcutaneous tissue were incised to reach the fascia surface and a suspension of *St. aureus* P-505 culture (2×10^6 cells/ml) was immediately applied. The treatment was started next day after the onset of the wound process. The wounds in the three test groups were treated with (i) a copolymer-based composition, (ii) a terpolymer based composition, and (iii) the reference ointment Levosin containing levomycetin (antibiotic), sulfadimethoxine (sulfanilamide), methyluracil (regeneration stimulant), and trimecaine (local anesthetic), based on a poly(ethylene oxide) mixture PEO-400 and 1500 ensuring the required osmotic activity. In the fourth (untreated control) group, the wound healing proceeded in the natural way. The efficacy of local treatment was judged using the following criteria: (i) character and expression of inflammatory reaction; (ii) terms of granulation development; (iii) onset time of epithelization; (iv) healing time.

Local irritant action of the co(ter)polymer hydrogels and related ointments was studied in a group of both male and female white mongrel rats weighing 170 – 220 g. Each test group involved ten animals. The gels and ointments (200 – 250 mg) were rubbed into the skin (deprived of hair) on an area of about 6 cm 2 on the side. This procedure was repeated daily over a period of seven days. During this period and shortly after, the animals were examined and their state was characterized in terms of the absence or presence of hyperemia, edemation, hemorrhage, infiltration, and desquamation (inflammation symptoms), the presence of which is evidence of the damaging (irritant) action on the skin [17].

Antimicrobial activity of the co(ter)polymer hydrogels and related ointments was studied *in vitro* with respect to the Gram-positive bacteria *St. aureus* and *B. subtilis* using the method of diffusion into agar. The cell cultures were grown on a liquid nutrient medium (meat – peptone broth,

TABLE 1. Osmotic Properties of Polymeric Ointment Bases

Composition	Ratio of components	Hygroscopicity, %	Swelling time, h
Copolymer (β-VEAA-AA)	7:93	1300	16
	10:90	1100	18
	17:83	900	24
Copolymer (β-VEAA-MAA)	10.5:89.5	Dissolution	10
	14:86	1990	12
	24:76	1830	18
Terpolymer (β-VEAA-NaMAA-MME)	26:63:11	12160	0.25
	29:51:20	Dissolution	0.25

pH 7.3 ± 0.2, T = 30 – 35°C, 18 – 20 h). The grown cultures were diluted (1 : 1000) in a sterile isotonic 0.9% NaCl solution, introduced into Petri dishes with the appropriate nutrient media, and spread to form a continuous layer over the surface. After drying, 6.0-mm-diameter wells were cut to which the aliquots (10 and 100 μg) of gels and ointments were introduced. The reference drug was lincomycin (10 μl of 0.25 mg/ml solution). The samples were incubated at 37°C for 24 h and then the growth of bacterial cultures was evaluated [18]. The antimicrobial properties were characterized by the diameter d (mm) of the zone of inhibited growth of test microbes. The zones with diameters below 10 mm and continuous growth were considered as the absence of antimicrobial activity; d = 10 – 15 mm was rated as weak activity; 15 – 20 mm, as moderate activity; and above 20 mm, as pronounced activity. Each substance was tested in three parallel runs.

RESULTS AND DISCUSSION

An important factor determining the properties of polymeric bases is their osmotic activity that accounts for the structure and mechanical properties of gels (ensuring normal drug transport under purulent wound conditions), the effective lysis of necrotic tissues and cleansing of wounds, and sorption of the wound discharge. Experimental data in the osmotic activity of the proposed polymeric bases are presented in Table 1. As can be seen from these data, the proposed compositions possess sufficiently high osmotic activity and can be used as bases for the ointments intended to heal purulent wounds in the first stage of the process, where the wound is filled with a purulent discharge. Introduction of the MME component increases the osmotic activity and reduces the swelling time from about one day to several minutes. However, a terpolymer containing about 20 mol.% MME loses the spatial network structure and cannot be used as an ointment base.

The maximum osmotic activity is inherent in hydrogels based on β-VEAA – MAA (14 : 86 mol.%) copolymer (CP) and β-VEAA-NaMAA-MME (26 : 63 : 11 mol.%) terpolymer (TP). These selected bases were used for the preparation of ointments. The polymers were swelled in a phosphate buffer solution at pH 8.2, since these conditions are optimum for the proteolytic activity of the immobilized alkaline protease [19].

The experimental ointments with biologically active components and emulsifier appear as viscous elastoplastic masses of a yellowish green color with a characteristic wormwood odor.

Wound-healing properties. The integral parameters of wound process in various test and control groups are summarized in Table 2. As can be seen from these data, both polymer-based ointment compositions and levosin produce a comparable influence on the terms of granulation development and the onset of epithelization, with a small advantage of the CP-based composition. A somewhat different pattern is observed for the terms of healing, where the TP-based composition reliably accelerates healing in comparison to both untreated control (by 11 – 15 days) and levosin (by 7 – 8 days). The period of healing with the CP-based composition amounts to 27 – 31 days, which is also reliably better than in the untreated control group.

Both polymeric complexes exhibited pronounced antiinflammatory and antimicrobial properties in stages I and II of wound development, where the therapeutic effect of the CP-based composition was slightly better than that of the TP-based ointment. This circumstance is probably related to the better drug release in the former case and the more favorable physicochemical properties (viscosity, adhesion, etc.) of the CP-based composition. However, on passage from stage II to III, the therapeutic effect of the TP-based composition becomes more pronounced, which can be explained by better film-forming properties.

Thus, it can be ascertained that the proposed CP- and TP-based compositions have a certain potential of wound-healing activity. In order to improve the achieved ef-

TABLE 2. Characteristics of Model Wound Development and Healing

Treatment	Time (days) to		
	onset of granulation	onset of epithelization	complete healing
CP-based ointment	5 ± 1	10 ± 1^	29 ± 2^
TP-based ointment	7 ± 1	12 ± 2	24 ± 2*^
Levosin	6 ± 1	11 ± 2	32 ± 2
Control	4 ± 1	17 ± 2	37 ± 2

Difference from * reference group and ^ control group are reliable for $P < 0.05$.

TABLE 3. Antimicrobial Activity (Inhibited Growth Zone Diameter, mm) of Polymeric Complexes and Related Ointments

Composition	Dose, μg	*S. aureus*	*B. subtilis*
Copolymer (β-VEAA-MAA)	10	continuous growth	continuous growth
	100	continuous growth	continuous growth
CP-based ointment	10	24 ± 0.6	21 ± 0.5
	100	no growth	no growth
Terpolymer (β-VEAA-NaMAA-MME)	10	continuous growth	continuous growth
	100	continuous growth	continuous growth
TP-based ointment	10	25 ± 0.7	20 ± 0.6
	100	no growth	no growth
Lincomycin	10 (0.25 mg/ml)	18 ± 0.4	23 ± 0.8

fect, it is possible either to increase the hygroscopicity of the polymeric complex or to reduce the content of biologically active components (since their high concentration damages the granulation tissue formed in stage II of the wound process).

Local irritant action. Hydrogels of the proposed co(ter)polymers and related ointments did not cause local irritation of the skin upon single or repeated application on the back of rats. No change in the skin color and evidence of edemation, hemorrhage, and desquamation were observed.

Antimicrobial properties. Data on the antimicrobial activity of the proposed polymeric complexes and related ointments are summarized in Table 3. As can be seen, the pure (drug-free) polymers did not exhibit antimicrobial properties, while both CP- and TP-based ointments in a dose of 10 mg per well showed a pronounced bacteriostatic effect, which is evidence of good drug release from the polymeric matrix. In a dose of 100 mg per well, both ointments fully inhibit the growth of test microbes.

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