Oxyferriscorbone: Ulcerostatic Effect and Its Possible Mechanisms

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It is found that oxyferriscorbone inhibits free-radical lipid peroxidation in blood plasma and gastric tissue. Interaction of the drug with modeled membranes is demonstrated. It is suggested that this effect underlies the mechanism of its ulcerostatic action.

Key Words: *oxyferriscorbone; membranes; gastric ulcer*

At present, oxyferriscorbone (OF), a stimulator of repair processes [8], is used for the treatment of gastric and duodenal ulcers. However, the specific mechanism of its therapeutic effect remains unclear. The development of an ulcer is accompanied by the destruction of tissues, in particular, cell membranes. In this connection it is of interest to study the effect of OF on membranes.

The aim of the present study was a comparative investigation of the effect of OF on the dynamics of healing of an experimental gastric ulcer and its interaction with membranes.

MATERIALS AND METHODS

The experiments were performed on nonpedigree albino rats weighing 160-180 g. The animals were divided into three groups: the first group were intact animals, the second comprised animals with ulcer treated with OF, and the third was the control group (animals with untreated ulcer).

Chronic gastric ulcer was reproduced under ether narcosis by the acetate method [14]. The experimental animals received OF intraperitoneally in a dose of 30 mg/kg for 10 days starting from the 1st day of the experiment. The animals were killed on days 3, 7, and 10 after the surgery, the stomach was incised, and the area of lesion was measured. The ulcerated area was subsequently excised and immersed in liquid nitrogen prior to analysis.

In both OF-treated and untreated animals 0.02 ml blood was drawn from the caudal vein before,

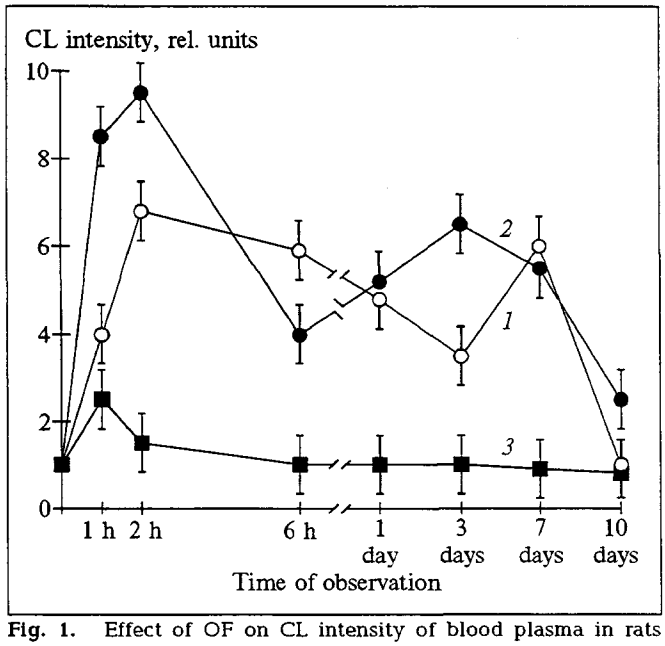

Fig. 1. Effect of OF on CL intensity of blood plasma in rats with experimental gastric ulcer. Here and in Fig. 2: *1*) CL in animals with untreated ulcer; *2*) CL in animals with OF-treated ulcer; *3*) CL in intact animals.

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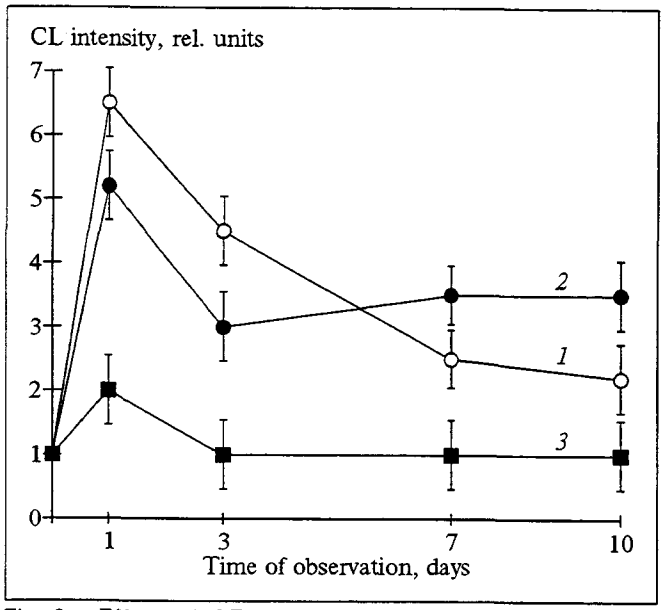

Fig. 2. Effect of OF on CL intensity in homogenates of ulcerated tissue in rats.

after 1, 2, 6, and 24 hours and 3, 7, and 10 days after surgery. The blood was mixed with 1 ml isotonic saline and centrifuged at 3000 g for 30 min, after which the plasma was drawn and used for subsequent analysis.

The intensity of free-radical lipid peroxidation (FRLP) was determined chemiluminometrically with an earlier-described setup [3]. FRLP was initiated by the addition of 10% H_2O_2 to plasma or 1 ml 10^{-2} M $FeSO_4$ to tissue homogenates. The antioxidative effect of OF was evaluated from the kinetics of all stages of Fe^{2+}-induced chemiluminescence (CL) in the system containing a suspension of multilayer liposomes prepared from chicken yolk. The ratio of the intensity of Fe^{2+}- or H_2O_2-induced to spontaneous CL, i.e., the burst amplitude, expressed in relative units, served as a measure.

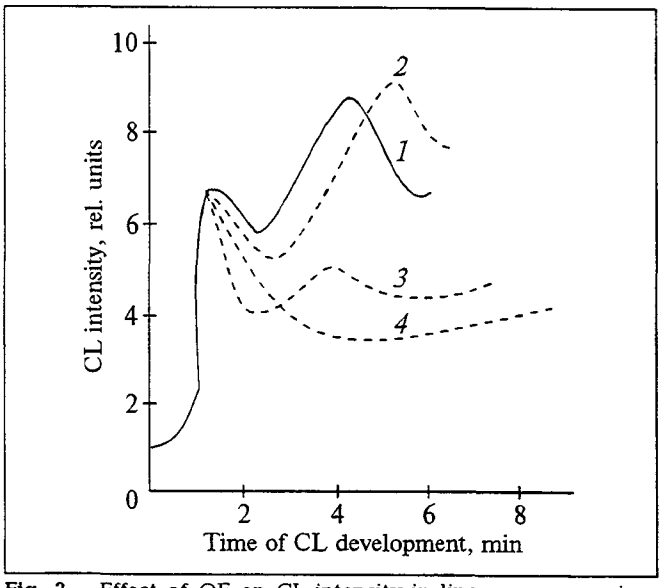

Fig. 3. Effect of OF on CL intensity in liposome suspension. *1*) control, *2*) 0.2 mg of the drug per ml suspension of liposomes; *3*) 0.6 mg per ml suspension; *4*) 1.0 mg per ml suspension.

Bilayer lipid membranes (LM) were prepared from a 4% solution of azolectine in n-decane [12]; liposomes were formed from chicken yolk as described previously [10]. Electroconductivity of LM was measured by the accepted method [5]. Viscosity and elastic properties of LM and their surface potentials were evaluated by analyzing the second and third harmonics of capacitance currents [9]. Microviscosity of liposomes was assessed by measuring the intensity of fluorescence of eximeric pyrene as described previously [4]. Na,K-ATPase activity was determined as described earlier [7] by the amount of inorganic phosphorus resulting from hydrolysis of ATP by ATPase. The activity of Ca^{2+}-ATPase and H^+-ATPase was determined potentiometrically [1,13]. Submitochondrial particles of myocardiocytes (SPM) were prepared by the method of ultrasonic disintegration [11] and their membrane potential was measured using ATP or sodium succinate as a substrate. The membrane potential of SPM was determined using phenyldicarboundecarborane, an artificial lipid-soluble anion, and an artificial phospholipid membrane as an ionselective electrode [6]. The activity of the adenylate cyclase system of reticulocytes was determined from the level of cyclic adenosine monophosphate (cAMP) [2].

The data were processed statistically using the Student *t* test.

RESULTS

The performed experiments indicated that OF markedly affected the healing of acetate-induced ulcer in rats. The data in Table 1 demonstrate that the drug considerably accelerates healing of the ulcer lesion ($p<0.01$).

In the plasma of untreated rats an activation of FRLP was found to occur, especially during the first 2 h after surgery, after which CL gradually decays (Fig. 1). In OF-treated animals the activation of FRLP was considerably lower in comparison with that in untreated rats during the same period ($p<0.01$). The study of FRLP intensity in the homogenates of ulcerated tissue showed that in untreated rats the intensity of CL increased and then decreased during the early stages of ulceration (the first 72 h after surgery), but it did not attain the normal level even by the 10th day postoperation. At the same time, the parameters of CL in

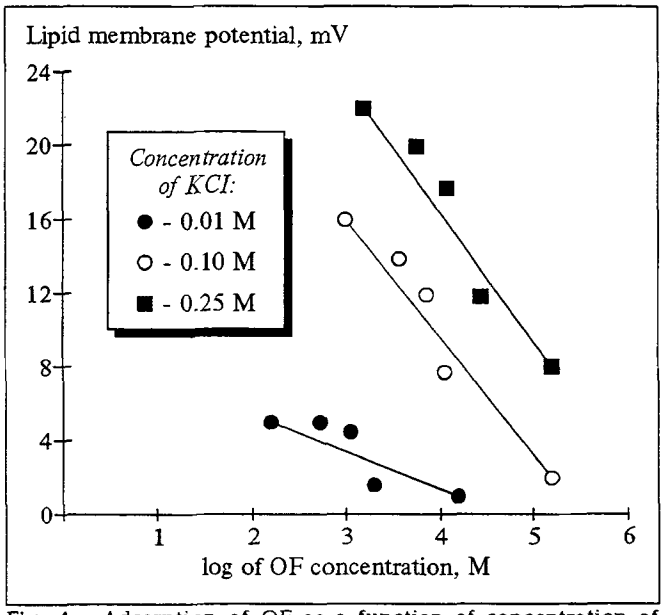

Fig. 4. Adsorption of OF as a function of concentration of KCl. *1*) concentration of KCl 0.01 M; *2*) 0.1 M; *3*) 0.25 M.

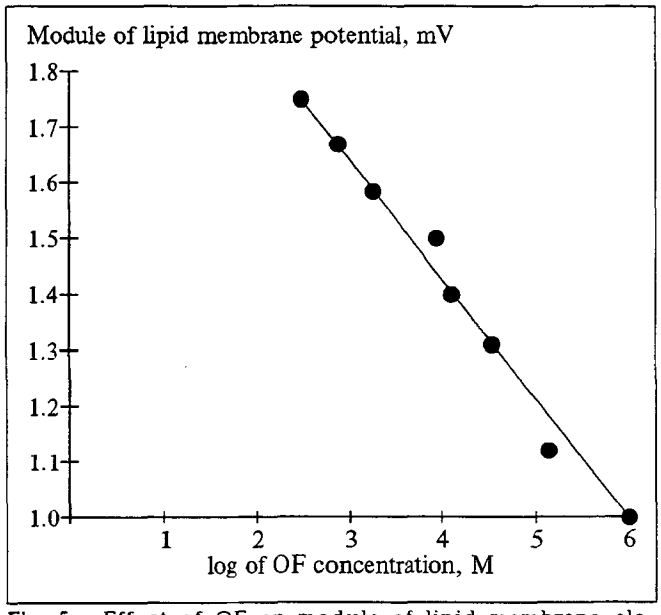

Fig. 5. Effect of OF on module of lipid membrane elasticity.

OF-treated rats virtually did not differ from those in intact rats (Fig. 2).

In vitro experiments on multilayer liposomes showed that the drug inhibits FRLP, which manifested itself in a decrease of parameters of Fe^{2+}-induced superweak luminescence. The effects of OF in doses of 0.2-1.0 mg per milliliter liposome suspension are presented in Fig. 3, which demonstrates the inhibiting effect of OF primarily in the phase of slow burst.

The measurement of the membrane potential of LM revealed that OF, upon being adsorbed on the membrane, increases its negative charge. This process depends on the ionic strength of the KCl solution (Fig. 4).

The study of the effect of OF on the viscoelastic properties of LM revealed that OF increases their module of elasticity (Fig. 5). The viscosity of liposome membranes was also affected by OF. For instance, microviscosity of the lipid bilayer was reliably decreased ($p<0.01$) within drug concentrations of 10^{-6}-10^{-8} M (Fig. 6).

The study of the effect of OF on the permeability of natural and modeled membranes showed that OF activates Na^+,K^+-, Ca^{2+}-, and H^+-dependent ATPases and the conductivity of LM. In parallel with this the decreased viscosity of the lipid bilayer after OF adsorption substantially (almost by 70%) enhanced the mobility of valinomycin.

The study of the effect of OF on the membrane potential of SPM showed that in doses of 20 and 50 mg/kg the drug does not affect the membrane permeability for protons, i.e., the electroinsulating properties of the membranes remain unchanged. Increasing the dose of OF to 100 mg/kg increased proton membrane permeability. However, this effect seems to be related to an overdose as well as to the fact that OF in this dose exerts a weak effect (and no effect at lower concentrations) on the level of cAMP in reticulocytes.

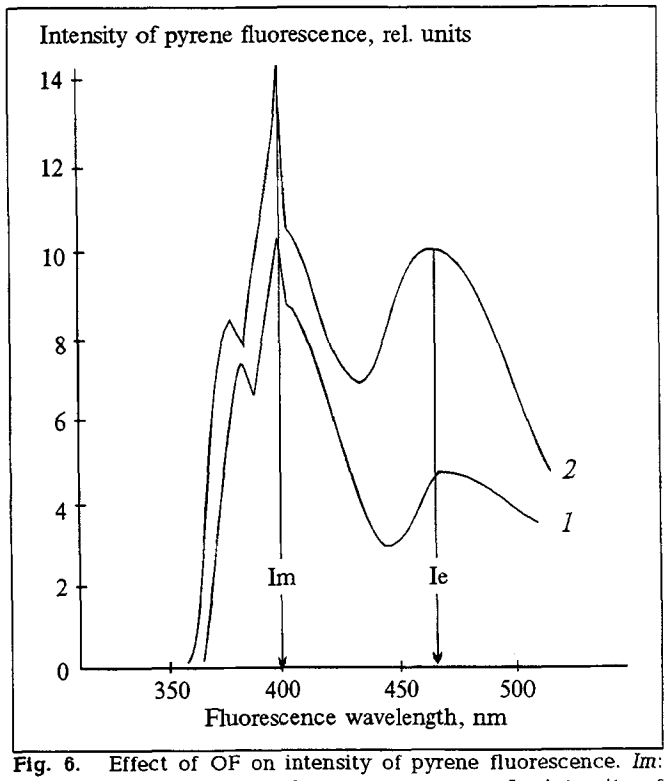

Fig. 6. Effect of OF on intensity of pyrene fluorescence. *Im*: intensity of fluorescence of pyrene monomers; Ie: intensity of fluorescence of pyrene eximers; *1*) control; *2*) spectrum in membranes with OF. Final concentration of drug 10^{-6} M.

TABLE 1. Dynamics of Area of Acetate-Induced Ulcer Treated with OF ($M \pm m$, $n = 7$)

Day of observation	Ulcerated area, mm²	
	untreated (control)	OF-treated (experiment)
3	31.8±3.1	21.7±2.5
7	28.6±2.2	7.8±0.8*
10	7.4±0.8*	0*

Note. An asterisk denotes $p = 0.01$ in comparison with the control.

The interaction of the preparation with cell membranes in the gastrointestinal tract is evidently mediated via an Fe atom which split off from the drug molecule during degradation. This atom presumably becomes incorporated in the lipoprotein membrane complex of gastrocytes, which on the one hand, steps up the membrane and cytoplasmic enzymatic process underlying the repair and, on the other, produces a protective effect by inhibiting the enzymatic reactions underlying the processes of degradation.

Thus, our experiments suggest that the ulcerostatic action of the drug is partially attributed to the membranotropic effect of oxyferriscorbone.

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