EMOXYPINE AS AN INHIBITOR OF ANGIOGENESIS

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Neovascularization is an important stage in the pathogenesis of inflammatory, neoplastic, and dystrophic processes. It plays a negative role in the survival of corneal grafts, in burns, and in various other eye diseases. Several substances with either effector or inhibitory activity on neovascularization are known. The former include heparin and copper compounds [6], the latter include protamine, cartilage extracts, etc. [7, 9, 10]. In clinical ophthalmology, in inflammatory and dystrophic processes, the drug emoxypine, a 3-hydroxypyridine derivative, has been used with good effects. It was synthesized by L. D. Smirnov and K. M. Dumaev [4], and possesses fibrinolytic [2] and antioxidant [3] activity, and has a protective action on the retina – hence its use in ophthalmology. It has been suggested that 3-hydroxypyridine derivatives can inhibit phosphodiesterase, and thereby change the levels of cyclic nucleotides, with which the ADP and ATP concentrations are linked in cascade fashion [3]. The aim of this investigation was to study the action of emoxypine on growing vessels in the rabbit cornea during aseptic inflammation and on the development of the vitelline vessels of chick embryos.

EXPERIMENTAL METHOD

To produce inflammation, a fragment of quartz measuring 1 × 1 mm was implanted in the deep layers of the cornea of both eyes of nine rabbits weighing 3.5-4.0 kg, at a distance of 3 mm from the limbus (Fig. 1a). The implants were sterilized beforehand in 70° ethanol for 15 min and washed in physiological saline. Daily 3 times a day 0.9% NaCl solution was instilled into one eye of each animal, and once a week this solution was injected beneath the conjunctiva (control), whereas 1% emoxypine was injected on the 4th-5th day in accordance with the same schedule (experiment). In series 1 (nine rabbits – 12 eyes) instillation of the solutions began immediately after the operation and these procedures were continued for 10-14 days. Every day the length of the ingrowing vessels was measured with the ocular micrometer of an MBS-1 binocular microscope. At the end of the period of observation the newly formed vessels were revealed by fluorescence angiography (FAG). For this purpose a 20% solution of fluorescein was injected into the rabbit's auricular vein in a dose of 0.07 ml/kg body weight and the vessels were photographed with the aid of a photographic slit lamp ("Opton," Germany) with standard filters. In the experiments of series 2, the control eyes from series 1, in which the cornea was abundantly vascularized (six animals – six eyes) were used as test objects. Emoxypine (1%) was instilled into these eyes 3 times a day during the next 10-14 days, after which the results were recorded visually. To study the action of emoxypine on the newly formed vitelline vessels, 0.1 ml of 1%, 0.5%, 0.7%, and 0.1% emoxypine (experiment, 11-12 embryos to each series) or 0.9% NaCl (control, 12 embryos), was instilled once into the chick embryos at the age of 48 h of incubation through a small window in the shell. The control and experiment were taken from the same batch of eggs. Emoxypine was diluted in physiological saline. The embryos were fixed at the stage of 72-74 h of incubation. To obtain total preparations the embryos together with the vascular field were spread out on a slide and fixed in 12% formalin solution for 20 min and then with 96° alcohol for

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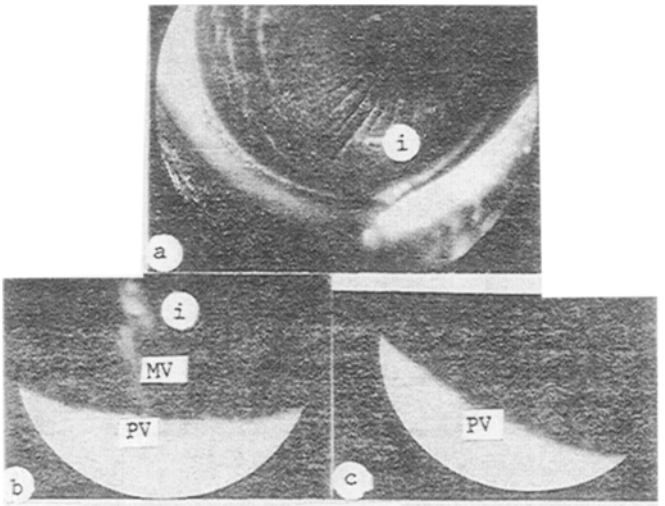

Fig. 1

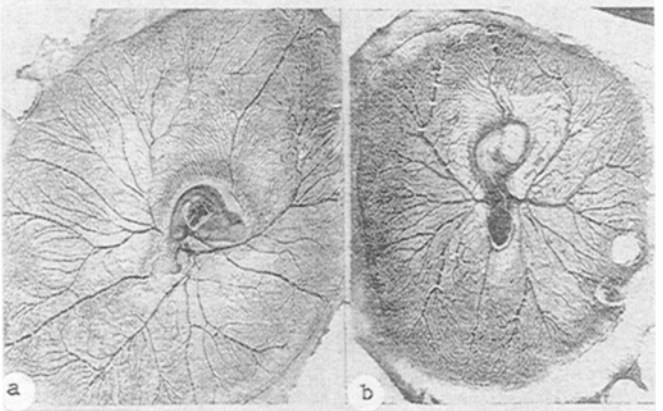

Fig. 2

Fig 1. Effect of emoxypine on corneal neovascularizztion: a) position of implant in cornea; b) newly formed vessels having reached implant in cornea of control eye. 12th Day after beginning of experiments. Vessels photographed after injection of fluorescein; c) absence of vascularization in experimental eye at same time. i) Implant, nv) newly formed vessels, pv) perilimbic vessels. 18×.

Fig. 2. Effect of emoxypine on vascular field of yolk sac of chick embryos: a) control, b) experiment.

20 min. The preparations were dehydrated and stained with Carazzi's hematoxylin. Measurements were made on undamaged preparations only. The total length and number of branches of the vessels of the yolk sac including large vessels of the I, II, and III orders, and also the area occupied by the embryo and the total area of the vascular field were measured by means of a "Mini-Mop" apparatus ("Opton," Germany), on photographs of the total preparations. Statistical data were obtained on a "Tg A 10" particle analyzer ("Opton") and subsequently analyzed by Kokitskii's method [5]. The significance of differences was assessed by Student's test.

EXPERIMENTAL RESULTS

In the experiments of series 1, on the 2nd day after implantation of quartz dilatation of the perilimbic plexus was observed in the sector opposite the implant Invasion of the cornea by blood vessels began on the 3rd-4th day

TABLE 1. Effect of 1% Emoxypine Solution on Chick Embryos and on Vessels of Yolk Sac

Object measured	Control (M ± m, n = 5)	Experiment (M ± m, n = 7)	T	Statistical significance
Number of brances of vessels of teh I, II, and III orders	100.2±10.6	59.2±7.7	3.040	$p<0.01$
Total length of vessels of I, II, and III orders, in conventional units	1440.9±75.6	1009.9±95.5	3.304	$p>0.01$
Area of vascular field, in conventional units	6308.9±150.2	5404.6±269.4	2.615	$p<0.02$
Area occupied by embryo, in conventional units	278.5±32.6	201.9±12.5	1.614	Difference not significant

after the operation in the control and on the 4th-5th day in the experiment. The daily growth of the vessels was 0.74 ± 0.08 mm in the control and 0.19 ± 0.1 mm in the experiment ($p > 0.01$). Toward the end of the 2nd week in the control the newly formed vessels had reached the implant, around which a capsule had formed (Fig. 1b). In three cases invasion of the cornea by blood vessels in the experiment was totally absent. In three other cases newly formed blood vessels were not observed for 1 month after cessation of emoxypine instillation. In three cases (six eyes), however, during the first 2 weeks, where retarded growth of the vessels took place, after cessation of emoxypine instillation growth of the newly formed vessels took place at the same rate as in the control.

The results of FAG of the anterior segment of the eye in the control revealed intensive filling of the perilimbic and limbic plexuses in the first phases of fluorescein application and irregular filling of newly formed vessels, running in the middle layers of the cornea and giving reticular branches in the capsule. The arterial and venous vessels could not be distinguished on the basis of their filling time. In the late phases of application, filling of the thin newly formed superficial vessels, creeping onto the periphery of the cornea, and also extravasation of fluorescein into tissue of the cornea and capsule were observed (Fig. 1b). The uneven caliber of the vessels and the extravasation are evidence of fenestration of the endothelium. In the experimental series, the results of FAG showed that the perilimbic and limbic plexuses were uniformly filled throughout their length and there was no extravasation (Fig. 1c).

In the 2nd series, after instillation of 1% emoxypine for 2 weeks, in two cases the vessels which previously invaded were first emptied and later disappeared completely In the remaining eyes only the small vessels disappeared. Thus the experiments showed for the first time that 1% emoxypine solution, if instilled into the rabbit's eyes in the presence of aseptic inflammation, can suppress the primary invasion of the cornea by blood vessels and give rise to reduction of newly formed vessels.

To determine how universal is the action of emoxypine, its action on the vitelline vessels of chick embryos was investigated in the course of formation (Fig. 2a). Quantitative evaluation of the results showed that 1% emoxypine solution, which is effective in suppressing growth of newly formed vessels in the rabbit cornea, had an inhibitory action on the vascular plexus of the yolk sac, where the total area of the vascular field and the length and degree of branching of the blood vessels were reduced (Table 1; Fig. 2b).

The 176 emoxypine solution had no action on the number of somites or the length of the embryo, All the lower emoxypine concentrations were ineffective. Toward the end of observation, in both experiment and control the embryos were at stages 17-18 of development (29-36 pairs of somites) [8]. It can be concluded from these results that the inhibitory action of 1% emoxypine solution is specific as regards suppression of blood vessel formation.

Emoxypine thus possesses inhibitory properties in relation to angiogenesis, The mechanism of this inhibition requires further study. The number of therapeutic preparations inhibiting the development of newly formed vessels is limited, and some of them (protamine), moreover, have a general toxic action [10]. In this connection the discovery of a nontoxic preparation with properties enabling its clinical use is encouraging.

REFERENCES

1. E. A. Egorov, A. A. Shvedova, and I. S. Obraztsova, Physiology and Pathology of the Intraocular Pressure [in Russian], Moscow (1987), pp. 78-86.

2. M. V. Zueva, A. A. Shvedova, and O. I. Shcherbatova, Vestn. Oftal'mol., No. 3, 56 (1977).
3. V. E. Kagan, N. H. Polyanskii, O. K. Muranov, et al., Byull. Éksp. Biol. Med., No. 4, 416 (1984).
4. E. K. Markovska and T. M. Volobueva, Republican Collection of Scientific Transactions [in Russian], Moscow (1990), pp. 89-92.
5. P. F. Rokitskii, Principles of Analysis of Variance for Biologists [in Russian], Minsk (1961).
6. C. Alessandry, K. Raju, and P. M. Gullino, Cancer Res., **43**, 1790 (1983).
7. S. B. Goren, R. Eisenstein, and E. Choromokos, Am. J. Ophthalm., **84**, 305 (1977).
8. V. Hamburger and M. L. Hamilton, J. Morph., **88**, No, 1, 49 (1951).
9. T. Matsubara, et al., J. Clin. Invest., **83**, No. 1, 158 (1989).
10. S. Taylor and I. Folkman, Nature, **197**, 307 (1982).