REFERENCES

1. V. L. Borovyagin, A. G. Sabelnikov, Y. S. Tarahovsky, and I. A. Vasilenko, *J. Membr. Biol.*, **100**, No. 3, 229-242 (1977).
2. J. F. Brandts, K. A. Lysko, A. T. Schwarts, *et al.*, *Colloq. Int. C.N.R.S.*, No. 246, 169-175 (1976).
3. R. B. Gun and R. G. Kirk, *J. Membr. Biol.*, **27**, No. 3, 265-282 (1976).
4. M. B. Kay, *Gerontology*, **37**, No. 1, 5-32 (1991).
5. J. Kurantsin-Mills and L. S. Lessin, *Pediatr. Res.*, **18**, No. 10, 1035-1041 (1984).
6. K. F. Lysko, R. Carlson, R. D. Taverna, *et al.*, *Biochemistry*, **20**, No. 19, 5570-5576 (1981).
7. L. Matovcik and W. Mentzer, *Clin. Haematol.*, **14**, No. 1, 203-221 (1985).
8. V. L. Shnyrov, S. N. Orlov, G. G. Zhadan, and N. I. Pokudin, *Biomed. Biochim. Acta*, **49**, No. 6, 445-453 (1990).
9. J. W. Snow, J. Vincentelli, and J. F. Brandts, *Biochim. Biophys. Acta*, **642**, 418-428 (1981).
10. K. T. Tokuyasu, R. Schekman, and S. J. Singer, *J. Cell Biol.*, **80**, No. 2, 481-486 (1979).
11. J. A. Ursitti, D. W. Pumplin, J. B. Wade, and R. J. Bloch, *Cell Motil. Cytoskeleton*, **19**, No. 4, 227-243 (1991).
12. G. Zhadan, E. Villar, and V. L. Shnyrov, *Biochem. Soc. Trans.*, **22**, No. 3, 386S (1994).

Experimental Validation for the Use of Recombinant Prourokinase and Its Immobilized Forms in the Treatment of Postoperative Fibrinoid Syndrome in Ophthalmology

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The specific therapeutic effectiveness of a single intravitreal injection of native and immobilized recombinant prourokinase is assessed in lensvitrectomized rabbits with the fibrinoid syndrome. The agents almost completely lyse the fibrin clot within 1-8 h. Heteroprotein conjugates of protein kinase with molecular weight of 900 kD and high fibrinolytic activity at 2-20-fold lower doses of the enzyme are preferable. Native and immobilized prourokinase are recommended for clinical use in case of intraocular fibrin formation after lensvitrectomy and other operations.

Key Words: *lensvitrectomy; intraocular fibrin production; prourokinase; immobilized fibrinolytic enzymes*

New-generation fibrinolytics — prourokinase (PU) and tissue plasminogen activator — have been recently developed and are now widely used in clinical practice [4,13]. Polymeric immobilized forms of the activators are preferable due to prolonged effect and the ability to accumulate in the pathological focus. They minimize the risk of side effects as a result of decrease in dosage and the number of drug injections at the same therapeutic effectiveness [3,10,12]. Fibrinolytics are widely used in ophthalmology for the treatment of intraocular hemorrhages and massive fibrin exudation after surgery [9,11]. The presence of the blood-aqueous barrier necessitates intraocular administration of the enzymes, which requires low drug doses and prolonged effect to prevent toxicity and repeated injections [1,2,6,8].

Our objective was to study the effectiveness of immobilized PU — soluble heteroprotein conjugates of a preset molecular weight and composition based

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on recombinant PU and oligomeric serum albumin (OSA) — in the treatment of the fibrinoid syndrome developing after cataract, vitreous body, and retina surgery. Tissue plasminogen activator has been used for this purpose [9,11], whereas the potentialities of PU and immobilized forms of the enzymes are still to be researched.

MATERIALS AND METHODS

The study was carried out on 16 Chinchilla rabbits (29 eyes) weighing 1.5 to 2.5 kg. After premedication (1 ml of 1% diphenhydramine intramuscularly), a rabbit was fixed on the operation table and narcotized with calypsol (30 mg/kg intramuscularly) and 1% procaine (1 ml retrobulbar injection). The pupil was dilated with 1% atropine sulfate and subconjunctival injection of 0.1 ml of 1% mesatone. The operation field was limited with the use of glove rubber (10×10 cm, a hole 10 mm in diameter), through which the eyeball was mobilized. Incisions were made on the conjunctiva and sclera at a distance of 1.5 mm from the limbus, and through these incisions tips of instruments were inserted in the eye: ultrasonic vitreophage and needle for liquid delivery. The lens was removed by phacoemulsification. Total vitrectomy was performed using a mechanical vitreotome. After the vitreous was removed, the liquid was replaced with gas by injecting a bubble of sterile air occupying 50-90% of the volume and the eye was hermetized by tying II-shaped sutures round sclerotomic holes. After the operation, 1 ml of 5% sisomycin sulfate was injected subconjunctivally. Laevomycetin (0.25%) and 1% atropine sulfate were daily instilled for 5 days postoperation.

Round or oval fibrin clot usually formed during the postoperative period. It was located near the pupil, and its size was expressed in arbitrary diameter (mm). If the clot was not round, a pair (or several pairs) of "diameters" were measured in perpendicular planes with a ruler and a slit lamp, and the mean arithmetic was calculated. The size of fibrin clot was measured on days 1, 2, 3, 5, 7, and 10 postoperation.

Fibrinolytic effect was studied after a single (on day 1 postoperation) intravitreal injection of recombinant PU with a molecular weight of 50,000 and specific activity of 130,000 IU/mg protein (Cardiology Research Center, Russian Academy of Medical Sciences) or soluble OSA-PU conjugates synthesized at the Institute of High-Molecular-Weight Compounds (Russian Academy of Sciences), using glutaraldehyde [5-7]. The OSA-PU conjugates exhibited 100% catalytic activity in comparison with intact PU, they had a mean molecular weight of 900 kD and equimolar composition, as estimated for

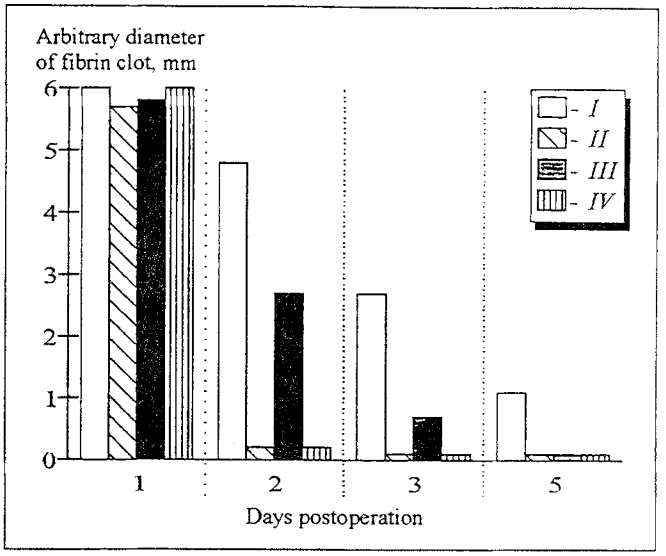

Fig. 1. Time course of lysis of intraocular fibrin clot after lens-vitrectomy in a rabbit. *I*) controls (n=12); *II*, *III*) prourokinase in doses of 500-1000 IU (n=6) and 100-250 IU (n=5), respectively; *IV*) heteroconjugates of oligomeric serum albumin and prourokinase with Mr 900 kD in a dose of 50 IU (n=6).

monomeric serum albumin and PU. The molecular weight of 900 kD was chosen previously as the optimal for intravitreal injection of OSA-PU [6].

The agents were dissolved in 0.9% NaCl solution to attain the needed concentration immediately before intravitreal injection of 0.1 ml. Controls (n=6) were either intact or intravitreally injected with 0.1 ml of 0.9% NaCl.

RESULTS

A round clot formed during the postoperative period in the lumen of the pupil. Sometimes, the clot was crescent-shaped and was located in the median and inferior compartments of the anterior chamber or looked like a semitransparent pupil membrane dividing the ocular cavity into two chambers. If the pupil lumen was completely blocked, ultrasonic B-scanning was employed. Fibrin clot never spread to the vitreous body and was located 1-2 mm beyond the iris on aphakic eyes. The clot was the largest on day 1 after surgery: in 12 control eyes its arbitrary diameter was 8 mm in 2 eyes, 7 mm in 2 eyes, 6 mm in 5 eyes, and 5 mm in 3 eyes. Later, the clot was spontaneously lyzed (Fig. 1); in control rabbits its lysis was completed on days 7-10. As fibrin disappeared, the pupil lumen was cleared, and the status of the vitreous chamber and retina was assessed by biomicroscopy and ophthalmoscopy. Gas was dissolved after 5-7 days, and the ocular cavity was filled with liquid which was slightly opalesced but then became absolutely transparent.

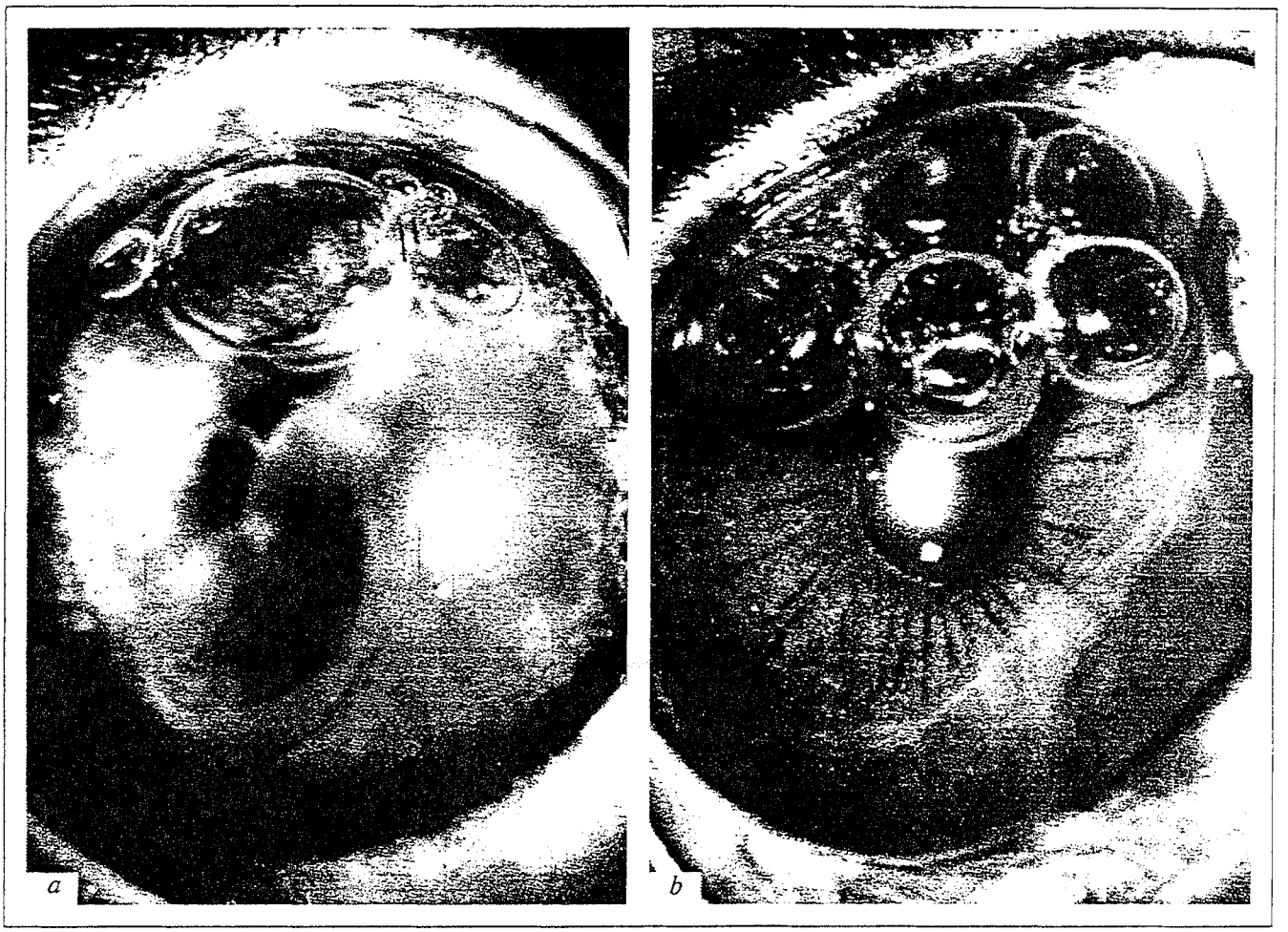

Fig. 2. Rabbit eye on day 1 after lensvitrectomy. *a*) fibrin clot 7 mm in diameter in the pupil zone and in the anterior chamber; *b*) complete lysis of fibrin in the pupil zone and its rudiments in the inferior compartments of the anterior chamber 6 h after intravitreal injection of 50 IU of conjugate of oligomeric serum albumin and prourokinase with Mr 900 kD.

The mean size of the clot in experimental groups did not differ from that in the controls on day 1 after surgery before injection of PU. The time course of fibrin lysis after injection is presented on Fig. 1. Intravitreal injection of native PU in doses of 500 to 1000 IU (effective in hemophthalmia, as reported previously [6]) resulted in virtually complete lysis of fibrin in 30-60 min after the injection. Biomicroscopy showed a slight increase in the opalescence of the intraocular fluid. On day 2, the irritation of the eye was the same as is common for the postoperative period, intraocular fibrin was seen in 2 cases (the mean arbitrary diameter of the clot was 0.5±0.3 mm). There were no relapses of fibrin production within 10 days. A decrease in the dose of native PU to 100-250 IU stimulated lysis of intraocular fibrin, but complete dissolving of the clot was slower: a 6-7-mm clot lyzed for 3-8 h. Relapses of fibrin production were observed on day 2 in all 5 cases, with the mean arbitrary diameter of the clot being 2.8±0.3 mm. New fibrin threads dissolved spontaneously and more rapidly ($p<0.05$) than in the control; by days 3-5 intraocular fluid was transparent. In the control, the mean diameter of fibrin clot at these terms was 2.7±0.2 and 1.5±0.2 mm, respectively. The fibrinolytic effect of PU in doses of 100 to 250 IU is evident, but such a dose is insufficient to prevent recurrences of fibrin formation.

Previously [1,6], we demonstrated a higher therapeutic efficacy of high-molecular forms of immobilized PU in hyphema and hemophthalmia due to prolongation of the fibrinolytic effect. Specifically, the use of OSA-PU with Mr 900 kD allowed us to decrease the dose of the enzyme injected in the vitreous chamber 50 times in comparison with native preparation used in the treatment of hemophthalmia.

Figure 2 shows the effectiveness of OSA-PU heteroconjugates in the fibrinoid syndrome following lensvitrectomy. Fibrin clot was almost completely dissolved 4-6 h after injection of 50 IU of OSA-PU,

i.e., in a dose lower than that of native PU. It should be emphasized that there were no relapses of fibrin exudation.

It is noteworthy that the incidence of postoperative complications (corneal opacities, hemophthalmia, detachment of the retina) was low and did not differ from that in the control animals.

Hence, intravitreal injection of PU in doses of 100 to 1000 IU and OSA-PU (Mr 900 kD) in a dose of 50 IU promoted lysis of intraocular fibrin clot after lensvitrectomy and arrested the fibrinoid syndrome within 1-2 days. Administration of PU and OSA-PU in the early postoperative period did not impair the cornea, induced no intraocular hemorrhages, and did not increase the incidence of detachment of the retina after vitrectomy. Intravitreal injection of lower doses of immobilized PU (2- to 20-fold in comparison with native preparation) provided a pronounced fibrinolytic effect without relapses of intraocular fibrin formation. Native and immobilized PU are recommended for prevention of the intraocular fibrin clot formation after lensvitrectomy and other intraocular operations.

REFERENCES

1. V. F. Danilichev, S. V. Kol'tsova, and E. V. Boiko, *Oftal'mol. Zh.*, No. 5-6, 303-307 (1993).
2. V. F. Danilichev and I. B. Maksimov, *Ocular Injuries and Diseases: Use of Enzymes and Peptide Bioregulators* [in Russian], Minsk (1994).
3. L. V. Zobina and A. D. Romashchenko, *Vestn. Oftal'mol.*, **103**, No. 1, 51-57 (1987).
4. G. P. Kleking and I. N. Bokarev, *Klin. Med.*, **68**, No. 6, 13-19 (1990).
5. S. V. Kol'tsova, *Bioorgan. Khimiya*, **21**, No. 6, 408-420 (1995).
6. S. V. Kol'tsova, E. V. Boiko, N. P. Kuznetsova, and V. F. Danilichev, *Vopr. Med. Khimii*, **41**, No. 3, 13-17 (1995).
7. N. P. Kuznetsova, R. N. Mishaeva, S. V. Kol'tsova, *et al.*, *Bioorgan. Khimiya*, **19**, No. 7, 871-879 (1993).
8. E. V. Chazov, R. A. Gundorova, A. D. Romashchenko, *et al.*, *Vestn. Oftal'mol.*, No. 4, 61-64 (1982).
9. G. J. Jaffe, G. A. Abrams, G. A. Williams, and D. P. Han, *Ophthalmology*, **97**, 184-189 (1990).
10. M. J. Poznansky, *Methods Enzymol.*, **137**, Pt. D., 566-574 (1988).
11. K. Schmitz, J.-H. Greite, and E. M. Bartenschlager, *Germ. J. Ophthalmol.*, **4**, 75-79 (1995).
12. V. P. Torchilin, A. V. Maksimenko, and A. V. Mazaev, *Methods Enzymol.*, **137**, Pt. D., 552-566 (1988).
13. M. Verstraete and D. Collen, *Blood*, **67**, 1529-1541 (1986).