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Improvement of ocular blood flow with dopamine antagonists on ocular-hypertensive rabbit eyes[1]

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ABSTRACT The eyedrops of the ocular-hypotensive dopamine antagonists, trifluperidol, moperone, lenperone, and spiperone, were instilled into an ocular-hypertensive rabbit eye. The blood flows in the choroid, retina, iris root-ciliary body, and iris were measured with colored microspheres at various time periods. It was found that all these dopamine antagonists, at a concentration of 0.5%, increased the blood flow in all eye tissues. Dopamine, at a concentration of 3%, produced a biphasic action by decreasing the blood flow initally at 30 min, then increasing it at 120 min and thereafter. But 1.5% dopamine produced a monophasic action which increased the blood flow after 180 min. Since dopamine anagonists are not cholinergics or adrenolytics, they are not supposed to produce the side effects induced by pilocarpine or timolol. It is hoped that they can become satisfactory drugs for glaucoma and ocular hypertension.

KEY WORDS ophthalmic solutions; dopamine; trifluperidol; moperone; lenperone; spiperone; blood flow velocity; ocular hypertension; retinal vessels; microspheres

Ocular blood flow, particularly in the retina and choroid, can be reduced as a result of ocular hypertenison, glaucoma, ischemic retinopathy, and the like[1-3]. Therefore, improvement of ocular blood flow in an ocular-hypertensive rabbit[3] with drugs could lead to the development of drugs that could be very useful for numerous eye diseases[3-6].

The majority of dopamine antagonists are known to reduce the intraocular pressure (IOP), although some are ineffective, and an even smaller number of them can cause ocular hypertension[7-11]. Trifluperidol, moperone, lenperone, and spiperone are dopamine antagonists known to lower the IOP[8,11]. They are tried on the ocular-hypertensive rabbits to see whether the blood flows in the retina, choroid, ciliary body, and iris can be improved. Dopamine has been reported to affect the IOP both ways[11]. Therefore, low (1.5%) and high (3.0%) concentrations of dopamine eyedrops were also instilled in the ocular-hypertensive eyes, and their effects on the ocular blood flow were studied.

MATERIALS AND METHODS

Materials Trifluperidol, moperone, lenperone, and spiperone were provided by Dr K Aimoto of Setsunan University, Japan. Dopamine was purchased commercially. Trifluperidol, moperone, and dopamine were dissolved in water; lenperone was dissolved in 80 % PEG 200 and 20% Me_2SO; and spiperone was dissolved in 60% PEG 200 and 40% Me_2SO. Eyedrops (25 μl) were instilled into rabbit eyes at time 0. Colored microspheres (15 μm in diameter) were purchased from E-Z Trac (Los Angeles CA). The colored microspheres were diluted with saline containing 0.01% (vol / vol) of Tween 80 to keep the microspheres from sticking together. One million microspheres in 0.2 ml were injected at each time point.

Rabbit for ocular blood flow New Zealand albino rabbits, weighing 2.5-3.0 kg, were anesthetized with ketamine 35 mg · kg^{-1} and xylazine 5

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mg · kg^{-1} intramuscularly. Half of the initial dose was given at 1-h intervals to maintain adequate anesthesia. An ocular hypertension was created by raising the IOP to 5.3 kPa (40 mm Hg) by inserting a needle directly into the anterior chamber of the eye, which was connected to a bottle containing an artificial aqueous humor solution. The surface of the solution in the bottle was raised to 54 cm (which was equivalent to 5.3 kPa) above the eye level, which reduced the ocular blood flow to approximately 1/3 of the normal values. The left ventricle of the heart was cannulated through the right carotid artery for microsphere injection, and the femoral artery was cannulated for blood sampling. The blood flow was measured with colored microspheres at −30 min for normal ocular blood flow and at 0 min for ocular blood flow with an IOP of 5.3 kPa. Trifluperidol, moperone, lenperone, spiperone, and dopamine eyedrops were instilled topically at time 0, and the blood flows were determined at 0, 30, 60, 120, and 180 min thereafter. At each injection of microspheres, blood samples were taken from the femoral artery for exactly 60 s immediately after the injection of the microspheres as a reference.

After the last injection of the microspheres and the collection of blood samples, the rabbits were euthanized. The eyes were dissected into the retina, choroid, iris, and iris root-ciliary body. The tissue samples were weighed, the blood samples were collected in heparinized tubes, and the volumes were recorded.

Counting of microspheres The sample processing and microsphere counting were provided by E-Z Trac. Briefly, tissue samples were added to Tissue / Blood Digest Reagent I in the microfuge tubes, sealed, and heated at 95℃ for 15 min. They were vigorously vortexed for 15–30 s, then reheated and revortexed until the tissue samples were all dissolved. Tissue / Blood Digest Reagent II was added while the samples were still hot, and the tubes were capped and vortexed. The tubes were centrifuged to settle the microspheres to the bottom of the tubes. The supernatant was aspirated, and the pellet was resuspended in the precise volume of Microsphere Counting Reagent with vortex. If the sediment aggregated, it was dispersed with an ultrasonic bath. The numbers of various colored microspheres were then counted with a hemocytometer.

Hemolysis Reagent was added to the blood sample which was then vortexed and centrifuged at 1500× *g* for 30 min. The supernatant was aspirated, and the Tissue / Blood Digest Reagent I was added. The following procedure was the same as that used to process tissue samples, and the colored microspheres were counted with a hemocytometer.

Calculation of blood flow The blood flow was calculated from the following equation:

$$Q_m = (C_m \times Q_r) / C_r$$

where Q_m is the blood flow of a tissue in terms of $\mu l \cdot min^{-1} \cdot mg^{-1}$, C_m is the microsphere count per mg of tissue, Q_r is the flow rate of blood sample in terms of $\mu l \cdot min^{-1}$, and C_r is the microsphere count in the referenced blood sample.

Statistical analysis All data were expressed as mean ± *s*. Group comparisons were made by *t* test.

RESULTS

With normal IOP, the blood flows in iris, iris root-ciliary body, retina, and choroid were determined ($n = 18$) to be 1.72 ± 0.36, 2.11 ± 0.38, 0.24 ± 0.038, and 13.29 ± 1.55, respectively. When the IOP was raised to 5.3 kPa, the blood flow was reduced to approximately 1/3 of the normal values, as shown in Tab 1.

When 25 μl of 0.5% trifluperidol were instilled into the eye, the blood flow in all tissues were increased (doubled) at 60, 120, and 180 min and reached the peak at 180 min.

In the case of lenperone, the results obtained were very close to those of trifluperidol, both qualitatively and quantitatively. However, it reached the peak action at 120 min.

Spiperone (0.5%) was a fast-acting drug reaching the peak of blood flow increase at 60 min and staying at plateau up to 180 min.

Moperone was a slow-acting agent increasing the blood flow significantly only at 180 min, although there was a tendency for

Tab 1. Blood flow ($\mu l \cdot min^{-1} / mg$ tissue) of ocular-hypertensive (IOP = 5.3 kPa) rabbits after drug instillation into eyes. $\bar{x} \pm s$. $^{*}P > 0.05$, $^{**}P < 0.05$ *vs* **0 min.**

Time / min	Iris	Iris root-ciliary body	Retina	Choroid
0.5% Trifluperidol, $n=6$				
0	0.53 ± 0.07	0.59 ± 0.07	0.062 ± 0.005	3.32 ± 0.51
30	0.70 ± 0.24*	0.83 ± 0.27*	0.083 ± 0.017*	4.29 ± 0.86*
60	0.88 ± 0.27**	1.06 ± 0.32**	0.11 ± 0.037**	6.39 ± 0.93**
120	0.78 ± 0.22**	0.89 ± 0.32**	0.091 ± 0.019**	5.37 ± 1.76**
180	1.10 ± 0.39**	1.32 ± 0.44**	0.13 ± 0.044**	6.80 ± 1.98**
0.5% Lenperone, $n=6$				
0	0.57 ± 0.17	0.66 ± 0.22	0.065 ± 0.010	3.49 ± 0.78
30	0.63 ± 0.15*	0.74 ± 0.22*	0.072 ± 0.010*	3.85 ± 0.81*
60	0.82 ± 0.19**	1.00 ± 0.32**	0.091 ± 0.17**	5.08 ± 1.13**
120	1.38 ± 0.24**	1.72 ± 0.32**	0.14 ± 0.027**	6.96 ± 1.18**
180	1.12 ± 0.29**	1.40 ± 0.42**	0.11 ± 0.029**	5.80 ± 1.40**
0.5% Spiperone, $n=6$				
0	0.54 ± 0.10	0.60 ± 0.10	0.062 ± 0.007	3.48 ± 0.78
30	0.59 ± 0.15*	0.68 ± 0.10*	0.070 ± 0.010*	4.09 ± 1.00*
60	0.94 ± 0.39**	1.07 ± 0.44**	0.11 ± 0.039**	6.47 ± 2.28**
120	0.92 ± 0.27**	1.01 ± 0.27**	0.098 ± 0.022**	6.11 ± 1.37**
180	0.93 ± 0.39**	1.02 ± 0.44**	0.10 ± 0.037**	6.24 ± 2.40**
0.5% Moperone, $n=6$				
0	0.60 ± 0.07	0.62 ± 0.07	0.063 ± 0.005	3.31 ± 0.37
30	0.61 ± 0.10*	0.65 ± 0.12*	0.069 ± 0.010*	3.53 ± 0.39*
60	0.77 ± 0.17*	0.84 ± 0.19*	0.085 ± 0.022*	4.45 ± 0.90*
120	0.74 ± 0.34*	0.82 ± 0.46*	0.074 ± 0.022*	3.74 ± 1.56*
180	1.21 ± 0.56**	1.41 ± 0.76**	0.14 ± 0.05**	7.51 ± 2.81**
1.5% Dopamine, $n=4$				
0	0.58 ± 0.08	0.61 ± 0.08	0.063 ± 0.006	3.07 ± 0.44
30	0.62 ± 0.08*	0.64 ± 0.08*	0.067 ± 0.006*	3.36 ± 0.82*
60	0.74 ± 0.22*	0.79 ± 0.18*	0.078 ± 0.016*	3.60 ± 0.58*
120	0.68 ± 0.14*	0.73 ± 0.08*	0.075 ± 0.010*	3.31 ± 0.46*
180	0.92 ± 0.28**	1.08 ± 0.42**	0.10 ± 0.032**	5.03 ± 1.44**
3% Dopamine, $n=6$				
0	0.62 ± 0.07	0.68 ± 0.07	0.065 ± 0.005	3.71 ± 0.39
30	0.49 ± 0.07**	0.53 ± 0.10**	0.049 ± 0.007**	2.47 ± 0.44**
60	0.72 ± 0.22*	0.78 ± 0.24*	0.070 ± 0.017*	4.38 ± 1.37*
120	0.92 ± 0.22**	1.03 ± 0.29**	0.11 ± 0.019**	6.27 ± 1.91**
180	1.19 ± 0.36**	1.32 ± 0.44**	0.14 ± 0.032**	8.19 ± 2.50**

blood flow increase at 60 and 120 min ($P > 0.05$).

When 25 μl of 1.5% dopamine were instilled into the eyes, a small increase of blood flow was seen in all eye tissues only at 180 min. In the case of 3.0% dopamine, the blood flow was decreased at 30 min, then increased at 120 and 180 min. This biphasic phenomenon was very similar to those observed with *L*-timolol eyedrops[3].

DISCUSSION

More and more people now realize that the measurement of retinal and choroidal blood flow in addition to that of IOP is essential for more accurate diagnosis and

treatment of glaucoma and ocular-hypertensive patients. The main reason for this development is that the change of blood flow may be independent of the change of the IOP[1-6]. Trifluperidol, lenperone, and spiperone are dopamine antagonists of the butyrophenone type and are reported to lower the IOP in rabbit eyes[11]. It is understandable that the ocular blood flow in the retina and choroid increased when these ocular hypotensive agents were instilled to the eye. Although moperone was reported to be a potent ocular hypotensive agent[7,11], its effect on ocular blood flow was much weaker (Tab 1) than trifluperidol, lenperone, and spiperone. These results indicate that there is no corelation between the potency of IOP-lowering effects and ocular blood flow-increasing action of dopamine antagonists.

It has been reported that a low dose of dopamine (1.5%) lowered the IOP, whereas the high dose of dopamine (3.0%) increased the IOP[11]. It is interesting to note that the ocular blood flow was increased by 1.5% dopamine but decreased initially by 3.0% dopamine, then increased later. The biphasic action of 3% dopamine on the ocular blood flow did not relate well to the monophasic ocular hypertensive action. Again, these results seem to indicate that the change of the IOP is unrelated to that of the ocular blood flow.

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