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Effect of Continuous Intravenous Infusion of Carteolol Chloride on Tissue Blood Flow in Rabbit Optic Nerve Head

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Purpose: To investigate the effect of an intravenous infusion of carteolol on tissue blood flow in the optic nerve head (ONH) of rabbits.

Methods: Rabbits received either a 3-week topical instillation, or a single intravenous injection (10, 20, 30 μg/kg) or a continuous intravenous injection (2.5, 5, 20, 40, 80 μg/kg per hour) of carteolol. The plasma carteolol level was determined by the gas chromatography negative-ion chemical ionization mass spectrometric method. The ONH blood flow was determined by the hydrogen clearance method.

Results: The plasma level of carteolol after a 3-week instillation was 5.55 ng/mL, and a continuous intravenous injection (5 μg/kg per hour) led to approximately the same plasma level. The continuous intravenous infusion of 5 μg/kg per hour of carteolol significantly increased the ONH blood flow compared to the controls from 30 minutes to 2 hours after the beginning of the infusion (n = 10). The mean blood pressure and intraocular pressure (n = 6) were not significantly changed during the continuous intravenous infusion of carteolol.

Conclusions: These results suggest that the plasma carteolol level in rabbits after long-term instillation can increase the ONH blood flow. We conclude that the increase resulted from a reduction in the vascular resistance in the ONH. **Jpn J Ophthalmol 1999;43:490–494** © 1999 Japanese Ophthalmological Society

Key Words: Blood flow in ONH, carteolol, continuous intravenous application, hydrogen clearance method, rabbit eyes.

Introduction

Intraocular pressure and disturbances of intraocular circulation, especially in the optic nerve head (ONH), appear to be involved in the pathogenesis and progression of glaucoma.[1] Because medications for glaucoma are usually administered for extended periods, their effects on the ONH circulation must be considered.

Carteolol hydrochloride and timolol ophthalmic solutions are nonselective beta-blockers widely used to treat glaucoma. It has been reported that carteolol prevents the progression of visual field disturbances in normal tension glaucoma.[2] Two reports by Tamaki et al[3,4] describe their effects on the ONH circulation. They used the laser-speckle method to determine the normalized blur (NB) value in the ONH, an index of ONH tissue circulation, and reported that the ONH blood flow was unchanged in the rabbit eye after a single instillation of carteolol, but was significantly increased in both the treated and control eyes after 20 days of instillation. After a single instillation of carteolol in the normal human eye, the

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NB in the ONH increased significantly in both the treated and control eyes. These findings suggest that the systemically absorbed carteolol increased the ONH blood flow.

Because the effect of carteolol ophthalmic solution on the ONH blood flow has been studied only by the laser-speckle method, despite its clinically important effects, it should be studied by another method, such as the hydrogen clearance method, a conventional method of determining blood flow. However, blood flow cannot be determined and compared by the hydrogen clearance method before and after long-term instillation because of the method's invasive nature. Therefore, we first determined the carteolol concentrations given intravenously that would produce almost the same carteolol plasma level as long-term instillation. Using this concentration, we then determined changes in ONH blood flow by the hydrogen clearance method.

Materials and Methods

Japanese male albino rabbits weighing 2.1–2.8 kg were handled in accordance with the ARVO Statement on the Use of Animals in Vision Research. In experiment 1, 27 rabbits were divided into groups of 3; in experiment 2, 20 eyes of 20 rabbits were divided into groups of 10; and in experiment 3, 12 eyes of 12 rabbits were divided into groups of 6. Before the experiments, we confirmed that the ocular fundus was normal by funduscopy in all rabbits.

Experiments 2 and 3 as well as experiment 1, except for long-term instillation, were conducted under general anesthesia, maintaining a stable depth by continuous injection of sodium pentobarbital (Nembutal; Abbott Laboratories, Chicago, IL, USA). The initial Nembutal dose was 0.6 mL/kg, followed by continuous intravenous injection at 0.2–0.3 mL/kg per hour using a syringe pump (Terufusion TE-311; Terumo, Tokyo). Carteolol (Otsuka Pharmaceutical, Tokushima) was dissolved in physiological saline (Otsuka Pharmaceutical) to make solutions of various concentrations.

Experiment 1: Plasma Carteolol Levels After Carteolol Application

Plasma carteolol levels were determined by gas chromatography–negative ion chemical ionization mass spectrometry (GC-NICI-MS) after a long-term, topical instillation of 2% carteolol to eyes twice a day at 9 A.M. and 5 P.M. for 3 weeks (group 1). Plasma levels were also determined after a single injection of carteolol (10, 20, and 30 μg/kg) into an ear vein (group 2), and also during a continuous injection of carteolol (2.5, 5, 20, 40, and 80 μg/kg per hour) (group 3) into an ear vein.

In the long-term instillation experiment, blood was sampled at 0.5, 1, 2, and 3 hours after the injection. In continuous-injection groups, blood was sampled at 0.5, 1, 1.5, and 2 hours after the beginning of injection. A blood sample (3 to 15 mL) was taken from a femoral artery, and the plasma was immediately separated by centifugation (3,000 rpm for 15 minutes) at 4°C. The samples were kept at under −80°C until determination of plasma carteolol levels. The method of determining plasma carteolol levels is detailed elsewhere.[5]

Experiment 2: Changes in ONH Blood Flow During Continuous Intravenous Carteolol Injection

The changes in ONH blood flow, blood pressure, and heart rate were determined every 30 minutes for 2 hours while carteolol (5 μg/kg per hour) or physiological saline (2 mL/hour) was continuously injected into an ear vein. Optic nerve head blood flow was determined using a hydrogen clearance flowmeter (RBF-222; Biomedical Science, Kanazawa). A hydrogen needle electrode (ON95-025; Unique Medical, Tokyo), 100 μm in diameter, was inserted from an entry point 3 mm posterior to the corneal-limbus through the vitreous body into the central part of the ONH (depth: 0.7 mm) while viewing with a vitrectomy lens. Procedures for determining tissue blood flow are described elsewhere.[6] Blood pressure and heart rate were measured using a noninvasive hemodynamometer (BP-98E; Softron, Tokyo).

Experiment 3: Changes in Intraocular Pressure During Continuous Intravenous Carteolol Infusion

While using the same anesthesia as in experiment 2, changes in the intraocular pressure were determined using an Alcon Applanation Pneumatonograph (Alcon Labs, Fort Worth, TX, USA) every 30 minutes for 2 hours; the same volume of carteolol or saline as in experiment 2 was continuously infused into an ear vein. No hydrogen electrode was inserted.

Statistical Analysis

Results are presented as mean ± standard error (SE). Data on blood flow, blood pressure, heart rate, and intraocular pressure were analyzed by analysis

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of variance (ANOVA) for repeated measurements and *t*-tests. $P < .05$ was considered significant.

Results

Experiment 1: Plasma Carteolol Levels After Carteolol Application

The mean plasma level of carteolol was 5.55 ng/mL after long-term instillation of 2% carteolol (Figure 2). Changes in the plasma carteolol level after intravenous injection showed a strong dose-dependency (Figures 1 and 2). After a single injection, the carteolol plasma levels increased and peaked within 30 minutes and then decreased over time at all doses (Figure 1). Even after the maximum dose of 30 μg/kg, the plasma carteolol level did not reach the 5.55 ng/mL level attained after long-term instillation.

With continuous intravenous injection, carteolol peaked at relatively higher doses at 1.5 hours but, at lower doses, the increase was more gradual and almost attained a constant level (Figure 2). At a dose of 5 μg/kg per hour, continuous intravenous injection of carteolol produced and maintained a plasma carteolol level similar to that after long-term instillation.

Experiment 2: Changes in ONH Blood Flow During Continuous Intravenous Carteolol Injection

Figures 3–5 show changes in the ONH blood flow, blood pressure, and heart rate during a continuous intravenous carteolol infusion (5 μg/kg per hour). Compared with controls, treated animals had significantly higher blood flow in the ONH from 30 minutes to 2 hours ($P < .01$; *t*-test) after the infusion began. Two-way interaction was significant by ANOVA for repeated measurements ($P = .0001$). The maximum increase was 14% at 60 minutes. The mean blood pressure in the treated animals tended to decrease throughout the experiment but was not significantly different from controls. Treated animals had significantly lower heart rate at 1 and 2 hours ($P < .05$; *t*-test) compared to controls. Two-way interaction was significant by ANOVA for repeated measurements ($P = .0250$).

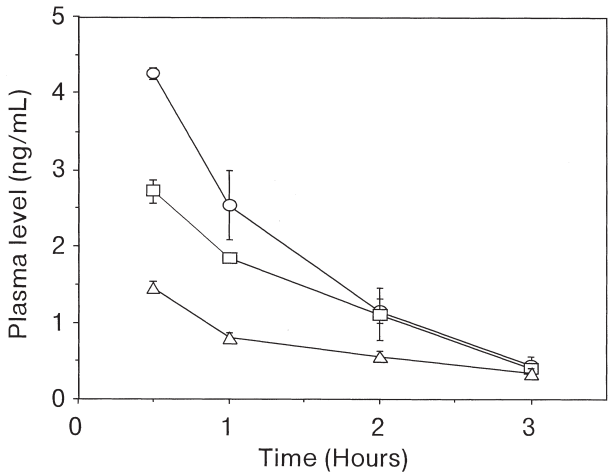

Figure 1. Changes in plasma level of carteolol after single intravenous injection of carteolol at 30 μg/kg (○), 20 μg/kg (□) or 10 μg/kg (△). Each plot represents mean (±SEM) of results obtained from 3 rabbits.

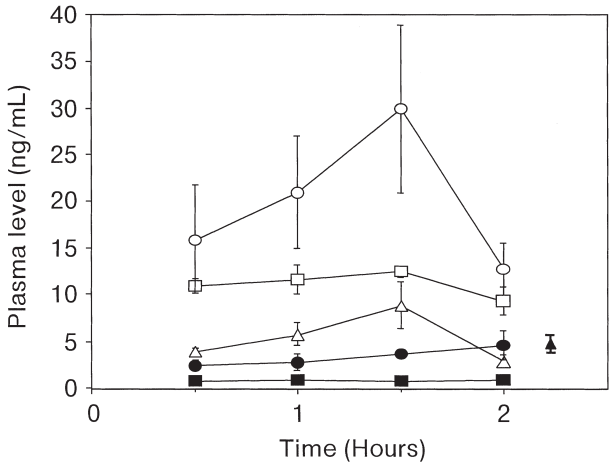

Figure 2. Changes in plasma level of carteolol after continuous intravenous injection of carteolol at 80 μg/kg per hour (○), 40 μg/kg per hour (□), 20 μg/kg per hour (△), 5 μg/kg per hour (●), or 2.5 μg/kg per hour (■), or after 3-week instillation of carteolol (▲). Each plot represents mean (±SEM) of results obtained from 3 rabbits.

Experiment 3: Changes in Intraocular Pressure During Continuous Intravenous Carteolol Infusion

Changes in the intraocular pressure during continuous intravenous carteolol infusion (5 μg/kg per hour) are shown in Figure 6. No difference was seen between controls and treated animals.

Discussion

GC-NICI-MS, used to determine plasma carteolol level, is highly sensitive in detecting unchanged carteolol in the plasma (because of carteolol's low molecular weight of about 300, biological components may interfere with carteolol determination). Carteolol was extracted from plasma and subjected to pentafluorobenzoylation (PFB) and *O*-dimethylethylsilylation (DMES) to make a PFB-DMES deriva-

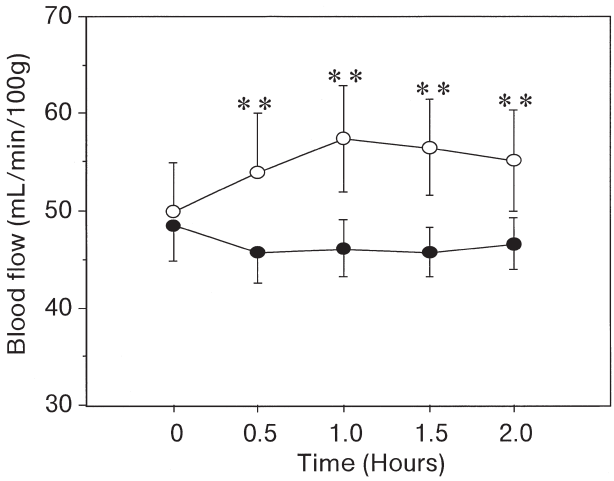

Figure 3. Changes in optic nerve head blood flow after continuous intravenous injection of carteolol at 5 μg/kg per hour (○) or physiological saline at 2 mL/hour (●). Each plot represents the mean (±SEM) of results obtained from 10 rabbits. Two-way interaction was significant ($P < .0001$; ANOVA for repeated measurements). Asterisks indicate significant differences from controls (**$P < .01$; Student *t*-test).

tive. The derivative was then detected by highly sensitive selected ion monitoring (SIM). The results showed that the determination was reliable in terms of the linearity of the calibration curve, reproducibility, specificity, and detection sensitivity (30 pg/mL).[5] After a single intravenous injection, plasma carteolol levels peaked within 30 minutes and then decreased over time. It did not continuously attain the same level as after a long-term instillation.

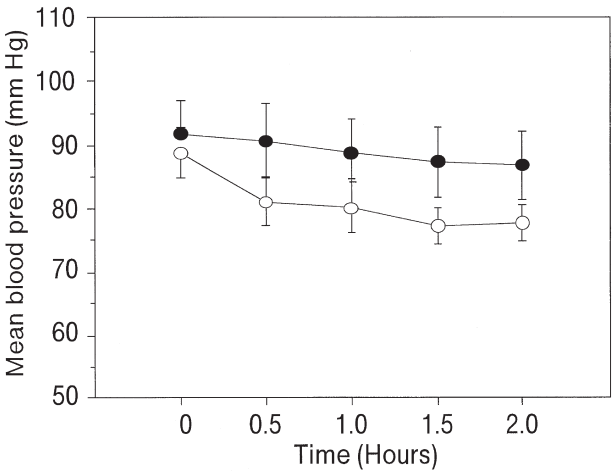

Figure 4. Changes in mean blood pressure after continuous intravenous injection of carteolol at 5 μg/kg per hour (○) or physiological saline at 2 mL/hour (●). There was no significant difference between these groups.

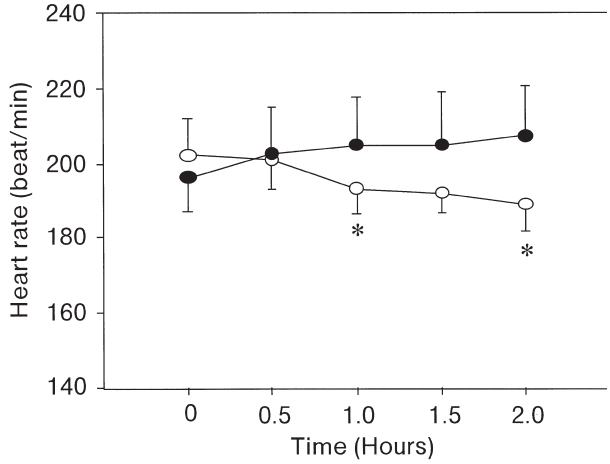

Figure 5. Changes in heart rate after continuous intravenous injection of carteolol at 5 μg/kg per hour (○) or physiological saline at 2 mL/hour (●). Two-way interaction was significant ($P = .0250$; ANOVA for repeated measurements. Asterisks indicate significant differences from controls (*$P < .05$; Student *t*-test).

During the continuous intravenous infusion, plasma carteolol levels increased and either peaked or plateaued at 1.5–2 hours. With an infusion concentration of 5.0 μg/kg per hour, the plasma carteolol attained a level comparable to that attained after a long-term instillation.

The changes in the ONH blood flow were thus determined with continuous intravenous injection at 5.0 μg/kg per hour. The hydrogen clearance method, used in determining ONH blood flow, is convention-

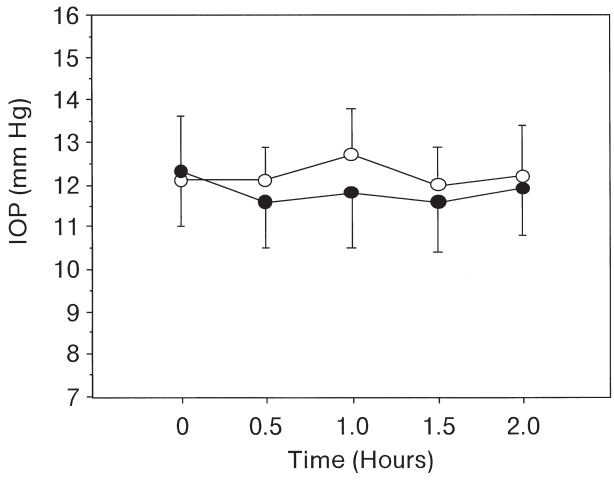

Figure 6. Changes in intraocular pressure after continuous intravenous injection of carteolol at 5 μg/kg per hour (○) or physiological saline at 2 mL/hour (●). Each plot represents mean (±SEM) of results obtained from 6 rabbits. There was no significant difference between these groups.

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ally used to determine tissue blood flow, and its quantitative precision has been demonstrated.[7] Although this procedure is somewhat invasive, no difficulties occurred in examining the effect of carteolol on tissue blood flow because the blood flow during the carteolol infusion was compared with that during physiological saline infusion.

Some studies have reported the effect of carteolol on ocular circulation: human retinal circulation was shown by laser Doppler flowmetry[8] to be unchanged by a single instillation of 1% carteolol, and pulsatile ocular blood flow in normal subjects was increased by a single instillation of 2% carteolol.[9] Only the two reports by Tamaki et al cover the effect of carteolol on the ONH.[3,4]

In our study, ONH blood flow in the rabbit's eye increased during the continuous intravenous infusion of carteolol at a dose producing almost the same plasma level as a long-term instillation. These results were compared with those of Tamaki et al who used the laser-speckle method.[3] According to them, long-term instillation significantly increased the NB in ONH in both eyes, and the increment was 16% for the treated eyes. Because changes of NB in ONH is correlated significantly with changes in ONH blood flow,[10] it was inferred that long-term instillation increased ONH blood flow by 16%. In our study, ONH blood flow increased by 14%, which is consistent with the results of Tamaki et al.

Vascular resistance (R) is calculated using Eq. (1)[11]:

$$R = OPP/F \quad (1)$$

where OPP = ocular perfusion pressure; F = ONH blood flow; and OPP is defined as in Eq. (2):

$$OPP = BP - IOP \quad (2)$$

where BP = mean blood pressure; and IOP = intraocular pressure.

Intraocular pressure was unchanged and mean blood pressure was unchanged or decreased, meaning that OPP was unchanged or decreased slightly. Accordingly, in Eq. (1), when F increases R should decrease, suggesting that the increased ONH blood flow is due to an action other than beta-blocking. Intrinsic sympathomimetic action (ISA)[12] of carteolol may decrease peripheral vascular resistance[13] or the stimulated secretion of prostacycline[14] may induce muscle relaxation in ONH. Janczewski et al[14] reported that carteolol at 10^{-8} to 10^{-7} M increased prostacycline secretion in vitro. Carteolol (MW: about 329) of 10^{-8} M is about 3.29 ng/mL. A level of 5.55 ng/mL was therefore likely to increase prostacycline secretion.

In this experiment, carteolol injection did not significantly change intraocular pressure. This may have been the case because the initial intraocular pressure, under general anesthesia, was about 12 mm Hg, which is relatively low and a further decrease was not possible.

In conclusion, carteolol may increase ONH blood flow in the rabbit eye by decreasing vascular resistance when plasma carteolol level is the same as that after long-term instillation.

References

1. Araie M, Sekine M, Suzuki Y, Koseki N. Factors contributing to the progression of visual field damage in eyes with normal-tension glaucoma. Ophthalmology 1994;101:1440–4.
2. Maeda H, Tanaka Y, Yamamoto M, Mizokami K. Effect of topical carteolol on visual function in normal-tension glaucoma. Nippon Ganka Gakkai Zasshi (J Jpn Ophthalmol Soc) 1997;101:227–31.
3. Tamaki Y, Araie M, Tomita K, Tomidokoro A. Effect of topical carteolol on tissue circulation in the optic nerve head. Jpn J Ophthalmol 1998;42:27–32.
4. Tamaki Y, Araie M, Tomita K, Nagahara M, Tomidokoro A. Effect of topical beta-blockers on tissue blood flow in the human optic nerve head. Curr Eye Res 1997;16:1102–10.
5. Nagasawa M, Kashimoto M, Sugawara M, Kimura Y. Determination of the beta-blocker carteolol in human plasma by a sensitive gas chromatographic-negative-ion chemical ionization high-resolution mass spectrometric method. J Chromatogr B 1995;673:294–8.
6. Sugiyama T, Azuma I. The effect of topically applied unoprostone on optic nerve head blood flow in circulatory disorder model eyes. Atarashii Ganka (J Eye) 1997;14:745–8.
7. Aukland K, Bower BF, Berliner RW. Measurement of local blood flow with hydrogen gas. Circulation Res 1964;14: 164–87.
8. Grunwald JE, Kostis J. Effect of topical carteolol on the normal human retinal circulation. Invest Ophthalmol Vis Sci 1992;33:1853–6.
9. Yamazaki S, Baba H. Acute effect of topical carteolol on ocular pulsatile volume change. Acta Ophthalmol 1993;71:760–4.
10. Sugiyama T, Utsumi T, Azuma I, Fujii H. Measurement of optic nerve head circulation: comparison of laser speckle and hydrogen clearance methods. Jpn J Ophthalmol 1996;40: 339–43.
11. Alm A. Ocular circulation. In: Moses RA, et al., eds. Adler's physiology of the eye. 9th ed. St Louis: CV Mosby, 1992:198–227.
12. Yabuuchi Y, Kinoshita D. Cardiovascular studies of 5-(3-tert-butylamino-2-hydroxy) propoxy-3,4-dihydrocarbostyril hydrochloride (OPC-1085), a new potent beta-adrenergic blocking agent. Jpn J Pharmacol 1974;24:853–61.
13. Man In't Veld AJ, Schalekamp MADH. How intrinsic sympathomimetic activity modulates the haemodynamic responses to beta-adrenoreceptor antagonists. A clue to the nature of their antihypertensive mechanism. Br J Clin Pharmacol 1982;13:245S–257S.
14. Janczewski P, Boulanger C, Iqbal A, Vanhoutte PM. Endothelium dependent effect of carteolol. J Pharmacol Exp Ther 1988;247:590–5.