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

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**Abstract**

**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.

**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 al³,⁴ 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...
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 centrifugation (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.

**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. Blood pressure and heart rate were measured using a noninvasive hemodynamic meter (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 Pneumatograph (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
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).

**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-
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). 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.

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-
ally used to determine tissue blood flow, and its quantitative precision has been demonstrated. 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 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. Only the two reports by Tamaki et al cover the effect of carteolol on the ONH. 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. 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, 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):

\[
R = \frac{OPP}{F}
\]  

(1)

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

\[
OPP = BP - IOP
\]  

(2)

where \(BP\) = mean blood pressure; and \(IOP\) = intraocular pressure.

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


