

Antithrombotic Effect of Ticlopidine in an Experimental Model of Retinal Vein Occlusion

Jorge G. Arroyo*, Kourosh Dastgheib[†] and Diane L. Hatchell[†]

**Massachusetts Eye and Ear Infirmary, Brigham and Women's Hospital, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA;* [†]*Department of Ophthalmology, Duke University Eye Center, Durham, NC, USA*

Purpose: Ticlopidine inhibits adenosine diphosphate (ADP)-induced platelet aggregation and may be effective in patients with retinal vein occlusions (RVO). This study tests the efficacy of ticlopidine in an animal model of RVO.

Methods: Rose bengal-mediated argon laser photothrombosis of retinal veins was created in rabbits pretreated with oral ticlopidine, aspirin, or saline. The number of laser spots necessary to produce a partial or complete RVO was recorded and tabulated.

Results: Pretreatment with ticlopidine significantly increased the number of laser spots needed to produce a partial ($P = .02$), or a complete ($P = .002$) RVO as compared to the control group. Pretreatment with ticlopidine significantly increased the number of laser spots needed to produce a partial RVO ($P = .02$). Pretreatment with aspirin significantly increased the number of laser spots needed to produce a complete RVO ($P = .002$).

Conclusion: Ticlopidine may be a useful antiplatelet agent for the treatment of patients with RVO. Patients treated with ticlopidine should be monitored for the possible development of hematologic disorders. **Jpn J Ophthalmol 2001;45:359–362** © 2001 Japanese Ophthalmological Society

Key Words: Aspirin, experimental retinal vein occlusion, laser photothrombosis, rose bengal solution, ticlopidine.

Introduction

Central retinal vein occlusion (CRVO) is a relatively common, often blinding, ocular thrombotic disorder for which there is no effective treatment.^{1–3} Histopathology of acute CRVO and branch retinal vein occlusions (BRVO) demonstrate disrupted endothelium and platelet/fibrin thrombosis within an often-narrowed vascular lumen. The fresh clot is usually found at the level of or behind the lamina cribrosa in CRVO or at an arteriovenous crossing in BRVO.^{4,5} The platelet/fibrin thrombus with time becomes stabilized and may become recanalized. Many treatments have been attempted to combat this dis-

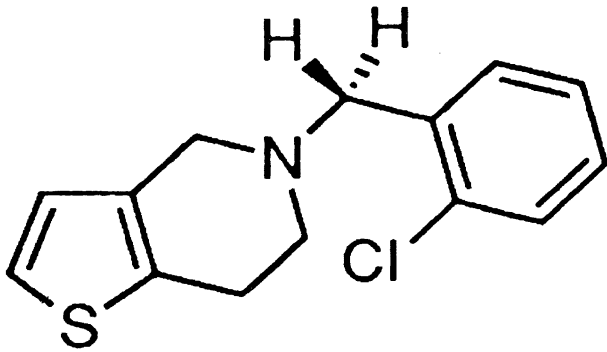
order with little or no success; these treatments include systemic fibrinolytics⁶ and anticoagulants,⁷ iso-olemic hemodilution,⁸ panretinal,⁹ grid,¹⁰ and chorioretinal photocoagulation,¹¹ hyperbaric oxygen,¹² and treatment with such antiplatelet drugs as bifemlane¹³ and troxerutin.¹⁴

Ticlopidine is a thienopyridine derivative that inhibits adenosine diphosphate (ADP)-induced platelet aggregation (Figure 1). The mechanism of action of ticlopidine is via indirect inhibition of fibrinogen binding to the glycoprotein IIb/IIIa complex.¹⁵ Ex vivo studies in rabbits have shown that ticlopidine significantly reduces the amount of platelet aggregation to extracellular matrix.¹⁶ In addition, ex vivo studies on human volunteers revealed a significant inhibition of ADP-induced platelet aggregation in the ticlopidine group versus placebo.¹⁷ A large multicenter clinical trial, the Canadian-American Ticlopidine Study, has demonstrated that ticlopidine in patients who have had a recent stroke reduced the incidence

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Correspondence and reprint requests to: Jorge G. ARROYO, MD, Department of Ophthalmology, Retina Service, 12th Floor, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114, USA



Ticlopidine

Figure 1. Chemical structure of ticlopidine.

of another stroke, myocardial infarction, or vascular death by 30.2% compared with placebo.¹⁸ Furthermore, ticlopidine has also been found to reduce complications due to platelet aggregation in patients undergoing hemodialysis, coronary artery bypass surgery, carotid endarterectomy, and extracorporeal circulation.¹⁹ However, oral ticlopidine is associated with diarrhea, rashes, hemorrhages, and hematologic disorders. Hematologic disorders such as leukopenia, thrombocytopenia, and pancytopenia are rare and resolve upon discontinuation of the drug, but close monitoring of the patients taking this drug is required.

We hypothesized that ticlopidine may be useful for prophylaxis, and also for possible treatment of a fresh platelet/fibrin thrombus in acute retinal vein occlusion (RVO). We tested this hypothesis in a platelet-rich model of RVO²⁰ in the albino rabbit by investigating whether pretreatment with ticlopidine or aspirin would increase the number of laser applications needed to produce a partial or complete occlusion of a retinal vein by photoactivation of intravenous rose bengal.

Materials and Methods

Ticlopidine (Hoffman-LaRoche, Nutley, NJ, USA) and aspirin (UDL Laboratories; Rockford, IL, USA) pills were separately ground into powder using a mortar and pestle and each dissolved in 1 mL of saline. Three hours prior to experimental retinal vein photothrombosis, 30 albino rabbits were orally fed 200 mg/kg ticlopidine (12 rabbits), or 200 mg/kg aspirin (7 rabbits), or 1 mL saline (11 rabbits). The animals were randomized (in order to mask the experimental status of the animal) prior to retinal vein

laser photothrombosis. All procedures involving animals conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Rose bengal-mediated argon laser retinal vein photothrombosis was used to produce focal RVOs within 1 disk diameter of the edge of the disk in one eye of each rabbit (Figure 2) Rose bengal dye was mixed with normal saline in a concentration of 20 mg/mL and filtered through a 22-micron filter. Forty milligrams per kilogram of the rose bengal solution was injected intravenously immediately prior to laser treatment. The argon laser was used to deliver a focal laser spot to the retinal vein (300 mW, 0.5 seconds, 50- μ m spot) every 5 seconds. The number of laser applications necessary to produce a partial or a complete RVO was recorded for each eye. Partial RVO was defined as the first sign of coating of the endothelium with a white plaque and narrowing of the blood column; complete RVO was defined as the first sign of complete interruption of the blood column by a white clot (Figure 3). The partial and complete occlusion results were tabulated for the ticlopidine, aspirin, and control groups. These groups were statistically analyzed using a two-tailed Student *t*-test with a level of significance of .05.

Immediately following these experiments, the animals were euthanized with an intravenous overdose of sodium pentobarbital (200 mg/kg) and the eyes removed and fixed in glutaraldehyde for transmission electron microscopy (EM).

Results

Transmission EM of the occluded retinal veins revealed a disrupted endothelium with multiple intrac-

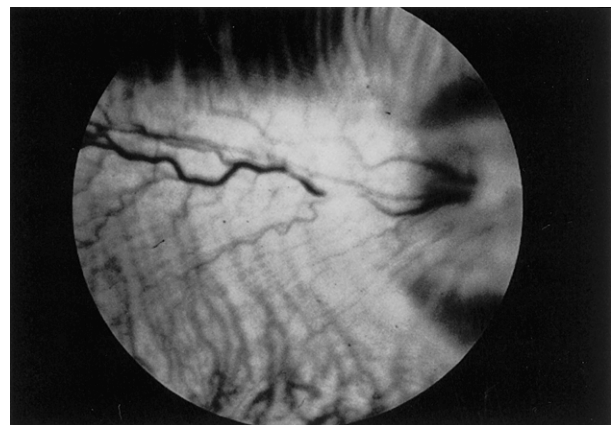


Figure 2. Complete rose bengal-mediated argon laser photothrombosis of albino rabbit retinal vein 1 disk diameter from optic nerve. Note dilated retinal vein distal to retinal vein occlusion.

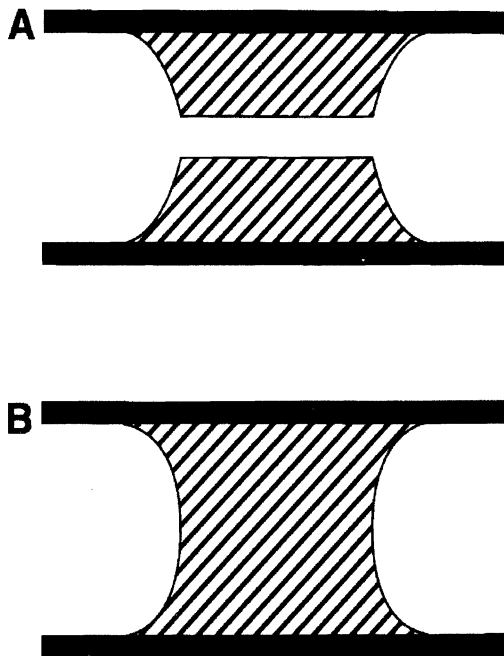


Figure 3. Diagram of partial (A) and complete (B) retinal vein occlusion.

cytoplasmic vacuoles. The lumen of the retinal veins were filled mostly with spherical degranulated platelets, small collections of trapped red blood cells, and a few white blood cells; no fibrin strands were observed in any of the sections (Figure 4).

The number of laser applications needed to create a *partial* RVO as compared to the control group (mean = 10.86) was significantly increased in the ticlopidine-treated animals (mean = 18.25; $P = .02$), but not in the aspirin-treated animals (mean = 17.71; $P = .19$) (Figure 5). The number of laser applications needed to create a *complete* RVO as compared

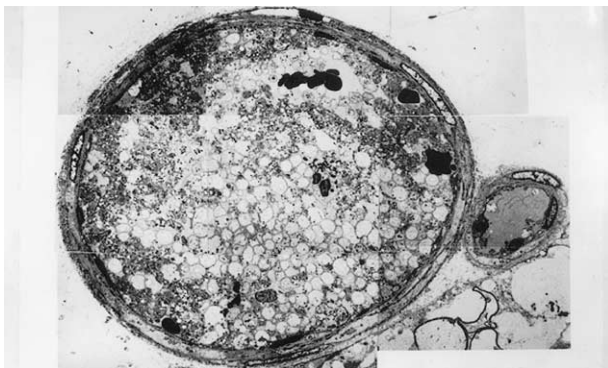


Figure 4. Electron micrograph of complete retinal vein occlusion. Note endothelial cell disruption and platelet aggregation within lumen of retinal vein.

to the control group (mean = 15.42) was significantly increased in both the ticlopidine (mean = 44.50; $P = .002$) and aspirin (mean = 30.57; $P = .02$) groups. Finally, pretreatment with ticlopidine significantly increased the number of laser applications needed to produce a *complete* RVO ($P = .02$) as compared to aspirin-treated animals. (Figure 6).

Discussion

Histopathological studies of acute RVO in humans reveal a disrupted endothelium and a platelet/fibrin thrombus at the site of vascular obstruction. In contrast, our animal model of RVO produced a platelet-rich clot associated with injured endothelium, but no electronmicroscopic evidence of fibrin. Rose bengal-mediated argon laser photothrombosis produces a clot via the photochemical production of free oxygen radicals, which damage the endothelium, causing rapid platelet aggregation and activation.¹⁹ This platelet-rich model of RVO is particularly suited to testing the *in vivo* efficacy of antiplatelet agents.

In our experiments, we recorded the number of laser applications necessary to produce either a partial or a complete RVO. A partial occlusion indicates how well platelets adhere to injured endothelium. In contrast, a complete RVO reflects how well platelets adhere to each other. Therefore, in these experiments, ticlopidine appeared to be more effective than aspirin in preventing platelet aggregation to the vessel wall (partial RVO) and platelet adhesion to other platelets (complete RVO). This potent antiplatelet action of ticlopidine may be due to its inhibi-

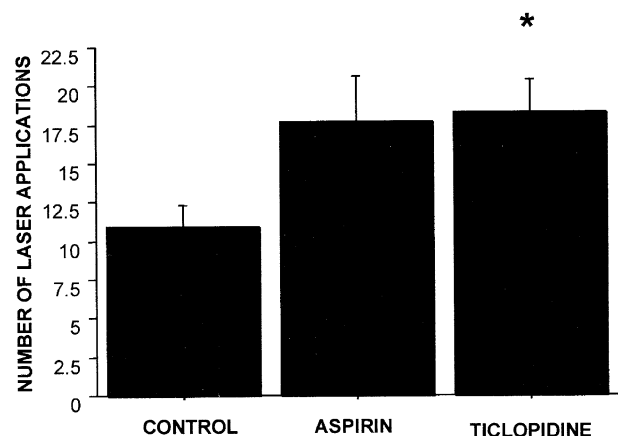


Figure 5. Graph of mean number of laser applications necessary to produce partial and complete retinal vein occlusion in albino rabbit. Error bars show \pm standard error of mean. One star indicates significantly different ($P < .05$) as compared to control group.

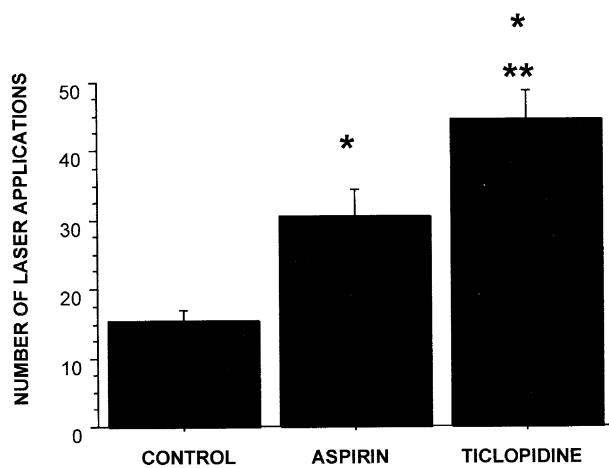


Figure 6. Graph of mean number of laser applications necessary to produce complete retinal vein occlusion in albino rabbit. Error bars show \pm standard error of mean. One star indicates significantly different ($P < .05$) as compared to control group. Two stars indicate significantly different ($P < .05$) as compared to aspirin group.

tory effect on the final common pathway for platelet-to-platelet aggregation, the IIb/IIIa receptor.¹⁵⁻¹⁹

Our results suggest that ticlopidine may be clinically useful for the treatment of the platelet component of RVOs. Specifically, ticlopidine may be effective prophylactically in patients who are at high risk for developing an ischemic RVO; patients with an RVO who have been successfully treated with local fibrinolytic therapy; patients with early stages of RVO, such as venous stasis retinopathy or non-ischemic CRVO; or patients with a history of RVO in the past who are at high risk of developing a new RVO in the same or fellow eye. However, patients treated with ticlopidine require close monitoring for such hematologic disorders as leukopenia, thrombocytopenia, and pancytopenia.

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