

Clopidogrel Use for Reducing the Rate of Thrombosis in a Rat Model of Microarterial Anastomosis

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Objectives: The national success rate for microvascular free tissue transfer is around 94%. However, in cases of failure, which is most often due to thrombosis in the vascular pedicle, the morbidity can be significant. Microvascular surgeons have used various pharmacologic agents to reduce thrombosis rates; however, none have been ideal. We examined the effects of clopidogrel bisulfate (Plavix), a member of the relatively new class of antiplatelet agents, on the rate of arterial thrombosis in a rat model.

Design: Prospective randomized, blinded.

Setting: Tertiary care academic medical center.

Subjects: Male Sprague-Dawley rats weighing between 350 and 400 g.

Intervention: Clopidogrel or placebo via gavage. After waiting 2 hours for absorption and activation, the "tuck" model of microvascular anastomosis was performed on both femoral arteries. Arteries were transected after 3 hours and patency was assessed.

Main Outcome Measures: Bleeding time was obtained by determining the time to clot after removal of 2 mm from the tail tip. Vessel patency was assessed after 3 hours in the clopidogrel-treated and control groups.

Results: Of 33 arteries, 19 (58%) in the control group developed complete thrombosis by the end of the period compared with only 6 (19%) of 32 arteries in the group that received clopidogrel. χ^2 Analysis revealed this to be significant ($P = .001$). The mean (SD) bleeding time in the control group was 158 (44) seconds compared with 233 (48) seconds in the clopidogrel group.

Conclusions: Clopidogrel significantly reduced the rate of arterial thrombosis in a rat model of microvascular repair. The average bleeding time in the clopidogrel group was prolonged, suggesting that absorption and activation occurred. These preliminary data suggest a potential role for clopidogrel in select high-risk patients undergoing microvascular free tissue transfer.

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MICROVASCULAR FREE tissue transfer revolutionized head and neck oncologic surgery. Lesions that were potentially unresectable owing to the resulting defect could now be reconstructed, returning both form and function. Free tissue transfer has the advantage of immediate reconstruction in a single-stage procedure. With increased experience, the current national success rate is approximately 94%.¹⁻³ The significant morbidity and possible mortality associated with flap failure encourages further improvements.

Most flap failures are attributable to thrombosis within the anastomotic vessels.⁴ Improvements in technique, pedicle geometry, and patient selection have lowered failure rates.⁵ In an effort to further increase success rates, surgeons have used

a myriad of pharmacologic agents. The ideal agent would be effective, be easy to administer, have minimal adverse effects, and be of low cost. Currently, none of the commonly used agents (dextran, aspirin, or heparin) fulfill these criteria (**Table 1**).

Clopidogrel bisulfate (Plavix; Bristol-Myers Squibb/Sanofi Pharmaceuticals Partnership, New York, NY) is a relatively new oral antiplatelet agent that inhibits adenosine diphosphate-induced platelet activation by irreversibly antagonizing the binding of this potent agonist to its receptor. Clopidogrel has shown efficacy in preventing thrombosis in clinical settings. The Clopidogrel Versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) study was a prospective, randomized, double-blind trial that showed the efficacy of clopidogrel in reducing the com-

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Table 1. Properties of Commonly Used Antithrombotic Agents in Microvascular Surgery

Agent	Route	Mechanism of Action	Adverse Effects
Aspirin	PO/PR	Arachidonic acid metabolism	Nonspecific inhibition, GI upset
Heparin	IV	Inhibits conversion of fibrinogen to fibrin	Continuous infusion, fluid load, HIT syndrome
Dextran	IV	Multiple effects on coagulation cascade	Noncardiogenic pulmonary edema, renal failure, anaphylaxis

Abbreviations: GI, gastrointestinal; HIT, heparin-induced thrombocytopenia; IV, intravenous; PO/PR, by mouth/by rectum.

bined risk of ischemic stroke, myocardial infarction, or vascular death when compared with aspirin.⁶ Adverse effects of clopidogrel were fewer than with aspirin and were primarily gastrointestinal discomfort and hemorrhage.

Clopidogrel has also been shown to decrease the rate of thrombosis in an animal model. Bernat et al⁷ created a deep medial injury to the rat carotid artery with electrical stimulation. Inhibition of thrombosis occurred in a dose-dependent manner with increasing inhibition seen up to a dose of 5 mg/kg.

Considering its improved efficacy over aspirin, which is routinely used by some microvascular surgeons in free tissue transfer, clopidogrel may be useful in the prevention of thrombosis in anastomotic vessels. Animal models are routinely used to investigate the efficacy of pharmacologic agents prior to clinical use. The 4 characteristics of the ideal animal model of microvascular anastomosis have been defined by Kersh et al.⁸ First, a permanent vascular modification should be made to provide a continued source of thrombogenesis. Second, the model should mimic clinical repair. Third, the diameter of the vessels should measure between 1 to 3 mm to allow for complete occlusion by a thrombus. Finally, thrombus formation should be gradual.

The “tuck” model of microvascular thrombosis was refined by Stepnick et al.⁹ This model creates a thrombogenic intimal flap within the lumen of the vessel (**Figure**). The introduction of adventitia into the lumen of a vessel has been theorized to be a possible cause of thrombosis in the microvascular pedicle. The procedure itself is technically simple and readily reproducible. Stepnick et al⁹ found that a 66% thrombosis rate in rabbit femoral arteries usually occurred within 5 to 15 minutes. This rate of thrombosis is amenable to pharmacologic manipulation, and the model has been applied by Hadlock et al¹⁰ to rats. The rat is chosen because it is routinely used by training microvascular surgeons to practice anastomosis, provides appropriately sized vessels, and is relatively inexpensive. In the present study, the “tuck” model of microvascular anastomosis was performed in rats to determine the efficacy of clopidogrel in reducing rates of arterial thrombosis.

METHODS

ANIMAL MODEL

Male Sprague-Dawley rats (Charles River Laboratories, Cambridge, Mass; weight range, 350-400 g) were used, following

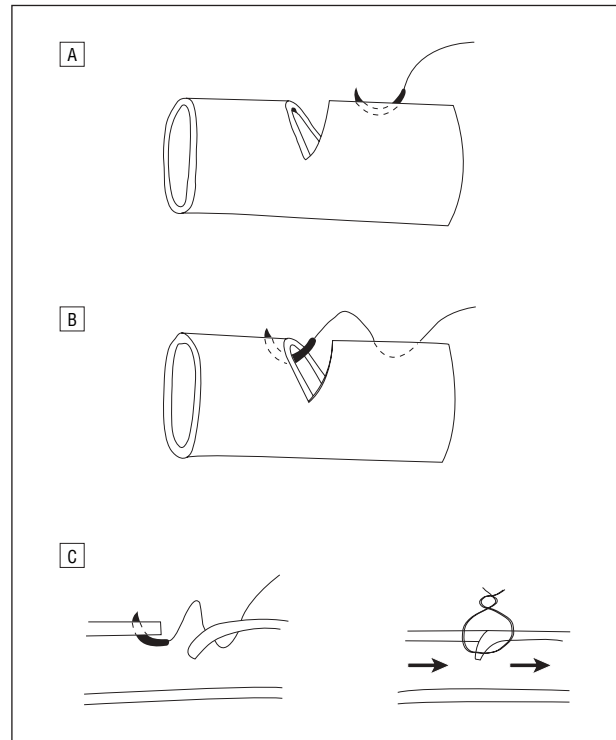


Figure. Schematic of “tuck” model of microvascular anastomosis.⁹ A, The suture is placed through the distal end of the vessel and brought out closer to the arteriotomy site, still on the distal side. B, The suture is then passed from within the lumen of the arteriotomy out through the proximal vessel wall. C, Cross section shows a “tuck” of vessel wall occurs on tying down suture.

institutional guidelines regarding animal experimentation. The rats were anesthetized with intramuscular lidocaine hydrochloride–ketamine hydrochloride (1:1). The femoral artery was isolated and cross-clamped, and 1% lidocaine hydrochloride was placed topically to prevent vasospasm. A 180° arteriotomy was then created. 10-0 Nylon monofilament suture was used to create a “tuck” of adventitia into the lumen of the vessel using the operating microscope and microvascular instruments as described by Stepnick et al.⁹ Briefly, the suture was placed through the distal end of the vessel and brought out closer to the arteriotomy site, still on the distal side. The suture was then passed from within the lumen of the arteriotomy out through the proximal vessel wall (**Figure**). Additional sutures were placed to assure hemostasis as necessary. The cross-clamps were removed, and the vessel was observed for thrombosis over the next 3 hours. Immediate patency of the vessel was determined by milking the blood out of the distal artery and assessing for refill. Any vessel not demonstrating refill after release of the cross-clamp was excluded. The contralateral femoral artery then underwent the same procedure. At the end of 3 hours, the vessel was transected and assessed for blood flow.

Table 2. Rate of Thrombosis in Rat Femoral Arteries 3 Hours After Undergoing Microarterial Anastomosis With the "Tuck" Model*

Group	Patent	Thrombosed	Total
Control	14 (42)	19 (58)	33 (100)
Clopidogrel	26 (81)	6 (19)†	32 (100)

*Data are given as number (percentage). Three vessels were excluded for excessive bleeding (2 from the control group and 1 from the clopidogrel group). Two vessels were excluded for no-flow immediately after release of clamps (both from the clopidogrel group).

† χ^2 Test, $P = .001$.

EXPERIMENTAL PROTOCOL

Two hours prior to surgery, half of the rats were administered a 5 mg/kg bolus of clopidogrel bisulfate dissolved in isotonic sodium chloride solution (pH 2) via gavage in a coded, blinded, randomized fashion. This is the lowest dose that has been shown to maximally prevent thrombosis.⁷ The other half received isotonic sodium chloride solution (pH 2) alone. Bleeding time was ascertained by removing the distal 2 mm of the tail and measuring the time until clot in 15-second increments, as previously described.¹¹ Statistical analysis was performed using χ^2 tests.

RESULTS

There was 1 death from anesthesia-related complications prior to surgery. Three vessels had excessive bleeding after release of the cross-clamps and were not included (2 in the clopidogrel group and 1 in the control group). Two vessels, both from the clopidogrel group, clotted immediately after release of the cross-clamps and were excluded. This left 65 vessels in the study. Of 33 arteries, 19 (58%) in the control group demonstrated thrombosis. Of 32 vessels, 6 (19%) in the clopidogrel group had thrombosis. χ^2 Analysis showed this to be significant ($P = .001$) (**Table 2**). The mean (SD) bleeding time in the control group was 158 (44) seconds compared with 233 (48) seconds in the clopidogrel group.

COMMENT

Clopidogrel is an antiplatelet agent that has been shown to prevent thrombosis in both clinical trials and animal models.^{6,7} The aim of the present study was to determine the efficacy of clopidogrel in reducing the rate of arterial thrombosis in a rat model of microvascular anastomosis.

The mechanism of action of clopidogrel is irreversible binding of the adenosine diphosphate receptor on platelets. Adenosine diphosphate is a potent activator of platelets and stimulates aggregation. Platelet aggregation is considered to be primarily responsible for arterial thrombosis.¹² Bernat et al⁷ caused electrical injury to the rat carotid artery and found an increasing reduction in rates of thrombosis up to a clopidogrel bisulfate dose of 5 mg/kg, which was the dose used in

the present study. To assure consistent, reliable dosing, the medication was administered by gavage. This required that the clopidogrel be administered in acidic solution because it is only freely soluble at a low pH. Once absorbed, the majority of the clopidogrel is activated by the liver within 2 hours.¹³

There was a significant reduction in the rate of thrombosis in the rat arterial "tuck" model with clopidogrel vs placebo (19% vs 58%; χ^2 test, $P = .001$). The "tuck" model of microvascular anastomosis satisfies the criteria stipulated by Kersh et al⁸ for an ideal animal model. The 58% rate of thrombosis in our control group compares favorably with the 66% rate that Stepnick et al⁹ found in the rabbit model. Prior to undertaking this study, we chose to exclude any vessels that had thrombosed immediately on releasing of the clamps because it suggests an error in technique. Of 67 evaluable vessels, 2 were excluded for this reason. Both were in the clopidogrel wing of the study.

Clopidogrel affects platelet function that can be assessed by bleeding time. Bleeding time was determined by a previously described technique that involved removing the distal 2 mm of the tail and measuring the time to clot.¹¹ The average bleeding time in the rats receiving clopidogrel was elevated when compared with controls (233 vs 158 seconds), suggesting both absorption and activation of the compound. The wide variation in bleeding times is attributable to the subjective nature of the test, which has led to its decreased use in clinical settings. Other methods of assessing platelet function include ex vivo aggregation studies, which require specialized equipment.

There are characteristics of clopidogrel that confer advantages over other prophylactic, antithrombotic, pharmacologic agents currently in use. The adverse effects of clopidogrel are primarily gastrointestinal upset and hemorrhage. The neutropenia that is seen with other antiplatelet agents, such as ticlopidine hydrochloride, is not found in clopidogrel.¹³ Although it requires oral administration, bolus dosing is possible and attains high levels within 2 hours of administration. It could be administered intraoperatively and have an almost immediate effect.

Although arterial thrombosis is a cause of flap failure, venous thrombosis is considered to be a more common cause. The slower flow rate in the venous system is believed to result in fibrin-rich clots.¹² Although clopidogrel is an antiplatelet compound, it has reduced venous thrombosis in a rabbit model.¹⁴ Further studies are under way to determine the efficacy of clopidogrel in reducing venous thrombosis in a microvascular model. Yet, clinical situations may arise in which a specific intraoperative issue with the arterial anastomosis is noted, which could increase the risk of thrombosis, such as intimal tearing, vessel mismatch, or another technical problem. In these cases, a prophylactic antithrombotic agent might be considered. This study demonstrates the efficacy of clopidogrel in preventing thrombosis in an arterial microvascular anastomosis at risk.

Currently, there is no standard of antithrombotic prophylaxis in the setting of free tissue transfer surgery. Numerous high-volume centers have differing

protocol varying from no prophylaxis to the use of dextran, aspirin, or heparin. This study sought to demonstrate the potential efficacy of clopidogrel in preventing arterial thrombosis compared with placebo. With this established, future study will evaluate the relative efficacy of this drug in comparison with other accepted antithrombotic agents.

In summary, clopidogrel has the potential to increase success rates in patients undergoing microvascular free tissue transfer. The desire to prevent thrombosis must be tempered by the knowledge that excessive anticoagulation could result in a hematoma or other significant complications. Because most patients may not require any pharmacologic intervention, by determining the characteristics of high-risk patients, the use of clopidogrel could be reserved for these situations.

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