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Effect of Oral Cilostazol on the Patency of Microvascular Anastomoses in Rats

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Abstract : **In order to evaluate the effect of cilostazol on patency of microvascular anastomoses, a double blind control study was performed on microanastomoses of the femoral artery in rats. Thirty female Spraque-Dawley rats were divided into two groups according to a blind protocol. One group (group A) was given cilostazol orally (10 mg/kg) 2 hours before the operation and daily for 7 days post-operatively. Another group (group B) received normal saline solution orally in the same amount and at the same time schedule as group A. The femoral artery anastomoses were performed under standard microvascular conditions. Seven days post-operatively, the patency of the arterial anastomoses was evaluated by Acland's test and histological study. The patency rate by Acland's test was 73% in group A, and 53% in group B. There was no significant difference between the patency rates of these two groups (p = 0.082). The area of the patency of the lumen by histological study was 61.67% in group A and 48% in group B. There was no significant difference between the groups in relation to patency of the microanastomoses (p = 0.381). There were no complications observed in this study.**

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เรื่องย่อ : ผลของยา Cilostazol ต่อการเปิดโล่งของหลอดเลือดบริเวณรอยต่อของหลอดเลือดขนาดเล็กในหนู

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รายงานนี้แสดงผลการศึกษาของยาไซลอสทาซอลต่อการเปิดโล่งของหลอดเลือดบริเวณรอยต่อของหลอดเลือดขนาดเล็กในหนู การทดลองใช้หนู Spraque – Dawley เพศเมีย น้ำหนัก 200 – 250 กรัม จำนวน 30 ตัว การศึกษาแบ่งเป็น 2 กลุ่ม กลุ่ม A ได้รับยาไซลอสทาซอล ขนาด 10 มิลลิกรัมต่อกิโลกรัม ก่อนผ่าตัด 2 ชั่วโมง และได้รับยาในขนาดเดิมวันละครั้งจนครบ 7 วันหลังผ่าตัด กลุ่ม B ได้รับน้ำเกลือในปริมาณเท่ากัน ทั้งก่อนและหลังผ่าตัดเหมือนกลุ่ม A ได้ทำการตัดต่อหลอดเลือดแดง femoral ข้างซ้าย เส้นผ่าศูนย์กลางโดยเฉลี่ย 0.77 มม. ตามหลักการต่อหลอดเลือดโดยวิธีจุลศัลยกรรม 7 วันหลังผ่าตัดได้ทำการตรวจดูการเปิดโล่งของรอยต่อหลอดเลือดโดยวิธี Acland และดูการอุดตันของหลอดเลือดโดยการตรวจรอยต่อหลอดเลือดด้วยกล้องจุลทรรศน์ พบว่าค่าเฉลี่ยของการเปิดโล่งของหลอดเลือดบริเวณรอยต่อเท่ากับ 61.67% ในกลุ่ม A และ 48% ในกลุ่ม B ความแตกต่างนี้ไม่มีนัยสำคัญทางสถิติ ($p = 0.381$) นอกจากนี้พบว่ากลุ่ม A มีอัตราการเปิดของรอยต่อโดยวิธีทดสอบแบบ Acland เท่ากับ 73% กลุ่ม B เท่ากับ 53% ความแตกต่างนี้ไม่มีนัยสำคัญทางสถิติเช่นกัน ($p = 0.082$)

INTRODUCTION

The advent of microsurgical techniques has offered thousands of patients the opportunity of free tissue transfer and replantation. Despite improvement in techniques and experiences gained in clinical microvascular surgery, small-vessel anastomotic thrombosis remains a significant problem.

The properties for an ideal antiplatelet agent should include 1) being effective orally or parenterally, 2) rapid onset of action, 3) rapid cessation of the effect, 4) no toxicity, 5) minimal adverse side-effects, 6) no cumulative effect, 7) predictable effect per dose, 8) no requirement for laboratory monitoring, and 9) low cost.[1] Numerous antiplatelet agents, including acetylsalicylic acid, have been used to reduce the incidence of vascular thrombosis by inhibition of secondary platelet aggregation. However, the production of one vasodilating agent known as prostacyclin is decreased by acetylsalicylic acid. In addition, acetylsalicylic acid ultimately produces systemic bleeding and gastrointestinal irritation. This study was undertaken to evaluate the effect of oral cilostazol (Figure 1) on the patency of microvascular anastomoses in rats. Cilostazol is a rapidly reversible phosphodiesterase inhibitor type 3 (PDEI3) that decreases primary and secondary platelet aggregation and does not interfere with prostacyclin[3] (Figure 2). Cilostazol has been recently approved by the Food and Drug Administration (FDA) for the treatment of intermittent claudication.[4] Quantitative bleeding time is not altered by cilostazol administration.[5] Common adverse events are headache, diarrhea, abnormal stools and dizziness. A dosage reduction in renally impaired patients is not necessary according to its pharmacokinetics and metabolites.[6]

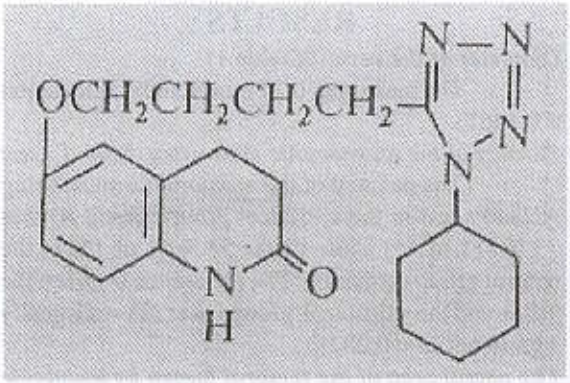

Figure 1. Chemical structure of cilostazol.

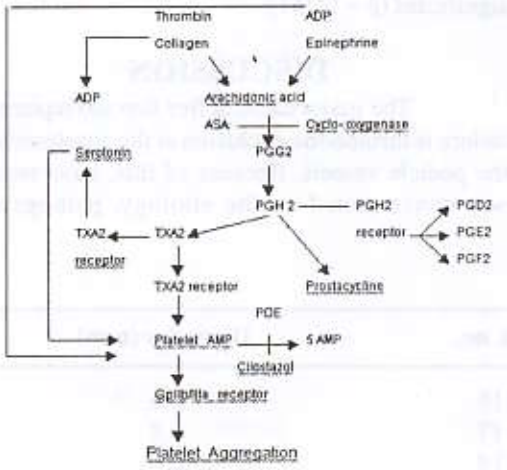

Figure 2. Mechanism of action of cilostazol.

MATERIALS AND METHODS

Female Spraque-Dawley rats weighing 200 to 250 grams (n = 30) were divided into 2 groups randomly. The blinded protocol using a single operative surgeon called for experimental animals to receive 10 mg/kg[7] cilostazol orally at 11.00 a.m. of operative day followed by cilostazol 10 mg/kg per day orally for 7 days (group A, n = 15). Control animals received an equivalent volume of saline (group B, n = 15). Sodium pentobarbital at a dosage of 50 mg/kg was administered to each rat as an anesthetic agent intraperitoneally.

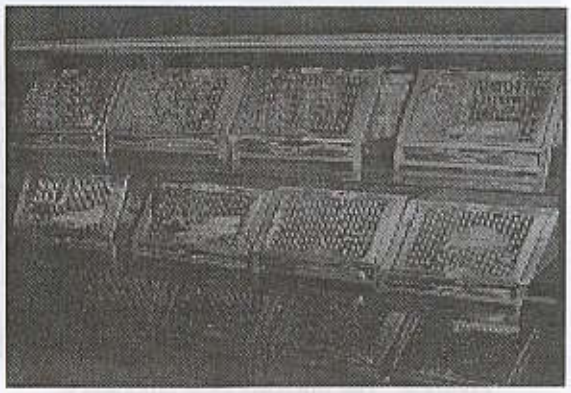

Figure 3. Each Spraque-Dawley rat was isolated in a cage.

Using an aseptic technique, the right common femoral arteries of all rats were cut with microsurgical scissors. The adventitia was stripped, excised and reanastomosed using a microvascular technique with interrupted stitches of 10-0 nylon suture (Ethilon 10-0, w2870). The groin incision was closed with interrupted stitches of 3-0 nylon suture. Seven days postoperatively, the animals were reanesthesized and the arterial anastomoses were evaluated by Acland's test and histologic study of the anastomotic sites. All animals were then sacrificed with intravenous KCl.

Acland's test was considered positive when there was some flow from the cut end of the vessel and negative when there was not.

Histologically, the percentage of the anastomosed vessel that was patent was evaluated from the most stenosed area cross-sectionally.

Fisher's Exact test was applied to determine any differences between group A and group B. It was significant if $p < 0.05$.

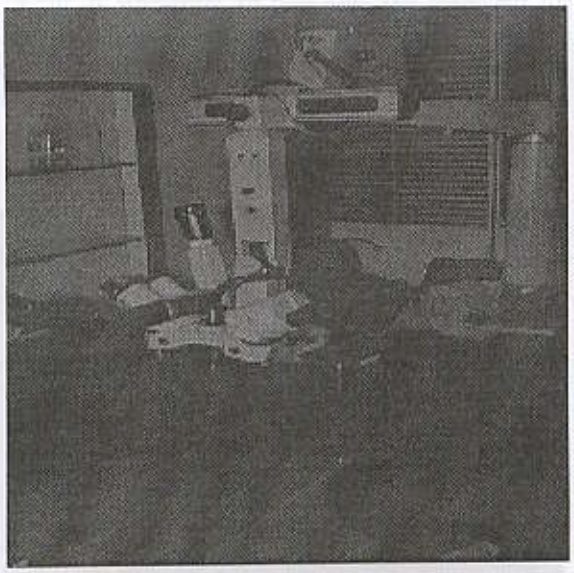

Figure 4. The microsurgical laboratory.

RESULTS

Diameter of the vessels (Table 1)

The mean was 0.77 mm (standard deviation = 0.1461).

Patency of the anastomotic sites using Acland's test

The patency of the anastomotic sites, using Acland's test in the cilostazol group (group A) was 73 % (11 of 15) while it was 53 % (8 of 15) in the control group (group B). The difference between the saline and the cilostazol groups was not statistically significant ($p = 0.082$).

***The patent area of the femoral lumen by histologic studies* (Table 2)**

The mean patent area in the cilostazol group (group A) was 61.67%, while in the control group (group B) it was 48%. The difference between the saline and cilostazol groups was not statistically significant ($p = 0.381$).

DISCUSSION

The major cause of free flap and replantation failure is thrombotic occlusion at the anastomoses of the pedicle vessels. Because of this, most research was concentrated on the etiology, pathogenesis,

Table 1. Diameter of femoral artery of each rat.

Rat no.	Diameter (mm)	Rat no.	Diameter (mm)
1	0.6	16	0.6
2	0.8	17	0.8
3	0.6	18	0.6
4	0.8	19	0.8
5	1	20	0.8
6	0.6	21	1
7	0.8	22	1
8	0.8	23	0.8
9	0.8	24	0.6
10	0.8	25	0.8
11	0.6	26	0.6
12	1	27	1
13	0.6	28	0.8
14	0.8	29	0.6
15	0.8	30	1
		mean	**0.77**

Table 2. The percentage of the femoral artery lumen that remained patent in each rat.

No.	Group A % of the patent area of the femoral artery lumen	Group B % of the patent area of the femoral artery lumen
1	0	100
2	20	50
3	0	20
4	100	85
5	0	0
6	100	90
7	85	60
8	100	10
9	100	0
10	0	75
11	50	0
12	100	30
13	85	100
14	100	100
15	85	0
Average	**61.67**	**48**

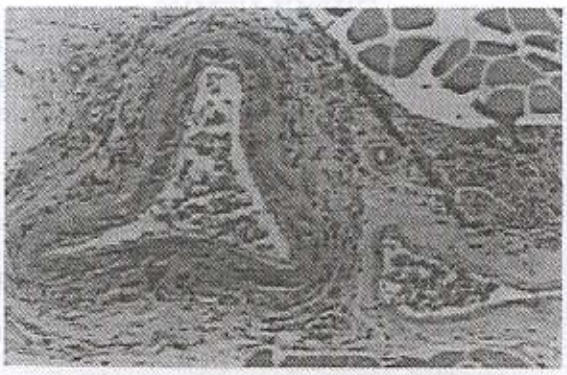

Figure 5. Patent vessel.

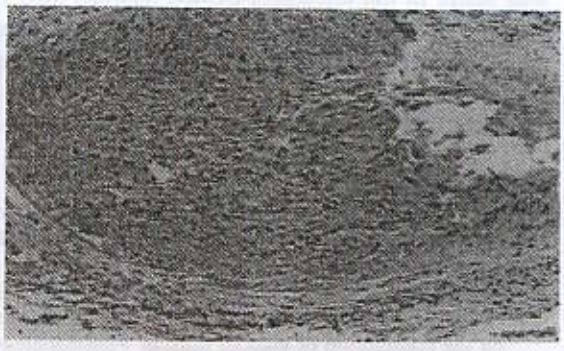

Figure 6. Occluded vessel.

therapy, consequences, and prophylaxis of thrombosis at the anastomotic site.

Recently, our clinical observations have led us to consider other possible antiplatelet agents such as cilostazol as an alternative to aspirin. Cilostazol has better properties than salicylic acid, such as a rapid onset (4 hours), rapid cessation of the effect (within 48 hours), more anti-platelet aggregation effect[5], minimal gastrointestinal irritation, a vasodilating effect (no interference with prostacyclin), inhibitory effect on arterial smooth muscle proliferation (via an increased level of

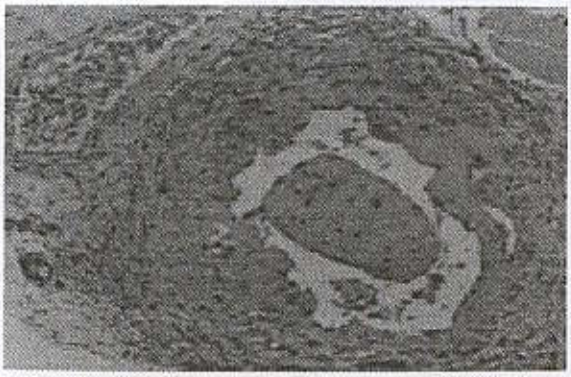

Figure 7. Thrombus size in patent vessel by Acland's test.

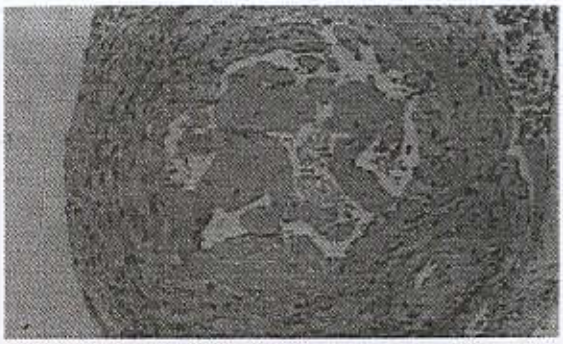

Figure 8. Thrombus size in occluded vessel by Acland's test.

intracellular cyclic AMP)[9] that may decrease the risk of microvascular anastomotic occlusion, no abnormal bleeding (no interference with bleeding time), and a reasonable cost. From Ohtake et al's study, cilostazol has been shown to produce an anti-thrombotic effect postoperatively.[10] This effect can prevent small vessels from occluding at the anastomotic site. Our data show that the patency of the anastomosed vessel was better in the cilostazol-treated animals than in controls and from the histologic study, the mean patent area was larger in the cilostazol treated group than in the control group.

However, both results showed no statistically significant differences. One of the failures to demonstrate significant usefulness in preventing anastomosis occlusion by cilostazol could be the small sample size of the study. Further study of a larger series is recommended.

CONCLUSION

Cilostazol, at an experimental dosage of 10 mg/kg/day in rats produced no statistically significant increase in patency of microvascular anastomoses.

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