Universidade de São Paulo

Biblioteca Digital da Produção Intelectual - BDPI

Departamento de Cirurgia e Anatomia - FMRP/RCA

Artigos e Materiais de Revistas Científicas - FMRP/RCA

2012

Effect of cilostazol and pentoxifylline on gait biomechanics in rats with ischemic left hindlimb

Effect of cilostazol and pentoxifylline on gait biomechanics in rats with ischemic left hindlimb

Matheus Bredarioli, MD,[a] Marcelo Bellini Dalio, MD, PhD,[a] Edwaldo Edner Joviliano, MD, PhD,[a] Willian Alves do Prado, MD, PhD,[b] and Carlos Eli Piccinato, MD, PhD,[a] *Ribeirão Preto, Brazil*

Objective: The purpose of this study was to determine the impact of pharmacologic treatment with cilostazol and pentoxifylline on gait biomechanics of ischemic rat hindlimbs compared with nonischemic controls.

Methods: An experimental study was designed using 30 Wistar rats divided into five groups (n = 6): control (C); ischemia (I) – animals submitted to left common iliac artery interruption without pharmacologic treatment; pentoxifylline (Pen) – rats submitted to procedure and treated with pentoxifylline 3 mg/kg twice a day for 6 weeks; cilostazol (Cil) – animals submitted to procedure and treated with cilostazol 30 mg/kg twice a day for 6 weeks; and sham (S) – animals submitted to procedure without artery interruption. Gait analysis was performed using a computed treadmill. Time, number, and duration of each hindlimb contact were obtained. The total number of contacts (TNC) and the total duration of contacts (TDC) were compared between left and right hindlimb and among groups. Left hindlimb ischemic incapacitation index (LHII) was defined by the formula:

$$LHII = \left(1 - \frac{TNC_{left} \times TDC_{left}}{TNC_{right} \times TDC_{right}}\right) \times 100$$

Results: Left hindlimb TNC values were twofold lower in I, Pen, and Cil groups than in C and S groups ($P < .01$). In I, Pen, and Cil groups, TNC values for the left hindlimb were half of the right hindlimb ones ($P < .01$). Left hindlimb TDC values were lower in I and Pen groups than the other groups ($P < .01$). Cil group presented twofold increased values, not different from C and S groups ($P = 0.16$). Right hindlimb TNC values were greater for I group ($P < .01$). LHII was around zero in C and S groups and 82 in both I and Pen groups ($P < .01$). Cil group presented a LHII of 42; higher than C and S groups, but lower than I and Pen groups ($P < .01$).

Conclusions: Cilostazol at a dose of 30 mg/kg twice a day promoted improvement in gait performance in rats submitted to chronic hindlimb ischemia. Pentoxifylline at a dose of 3 mg/kg twice a day did not show this effect. (J Vasc Surg 2012; 56:476-81.)

Clinical Relevance: Two medications are mostly used for treatment of intermittent claudication: cilostazol and pentoxifylline. Changes in gait biomechanics have been poorly documented as a result of pharmacologic treatment in these patients. Established experimental studies with animal models of ischemia described effects of medications on maximal distance of deambulation. No study used analysis of gait biomechanics as parameter of evaluation of the effect of pentoxifylline and cilostazol on intermittent claudication. The purpose of this study was to determine the impact of pharmacologic treatment with cilostazol and pentoxifylline on gait biomechanics of ischemic rat hindlimb compared with nonischemic controls.

Intermittent claudication (IC) is a common symptom experienced by patients with peripheral arterial disease (PAD).[1] It has been shown that IC is associated with alterations of gait characteristics.[2]

Two medications are mostly used for treatment of intermittent claudication secondary to PAD: cilostazol and pentoxifylline.[3,4] Although the purpose of these medica-

tions is to eliminate symptoms and improve the distance walked by patients with symptomatic PAD, changes in gait biomechanics have been poorly documented as a result of pharmacologic treatment in PAD patients.

Established experimental studies with animal models of ischemia described effects of medications on maximal distance of deambulation.[5,6] Gait biomechanics was altered in rats submitted to limb ischemia.[7] However, no study used analysis of gait biomechanics as a parameter of evaluation of the effect of pentoxifylline and cilostazol on IC.

The purpose of this study was to determine the impact of pharmacologic treatment with cilostazol and pentoxifylline on gait biomechanics of ischemic rat hindlimb compared with nonischemic controls.

METHODS

Animals. Thirty male Wistar rats (University of São Paulo, Ribeirão Preto, Brazil), weighing 100 to 150 g and clinically healthy were used for this study. Animals were

maintained in a room controlled for temperature and light and were provided food and water *ad libitum*. Animal care complied with the Principles of Laboratory Animal Care (formulated by the National Society for Medical Research) and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington, DC, National Academy Press, 1996). All protocols were approved in accordance with the Animal Experimentation Ethics Committee at University of São Paulo–Ribeirão Preto, Brazil.

To become acclimated with the treadmill protocol, animals were subjected to progressive training for 3 consecutive days. On the first day, animals were set on the treadmill without movement for 10 minutes. On the second day, they were exercised for 10 minutes with progressive speed ranging from 5 to 12 m min^{-1}. On the third day, they were exercised with speed of 12 m min^{-1} and preoperative gait recordings were obtained. Rats that did not adapt to this protocol were excluded (33%).

Experimental design. After the treadmill training, animals were randomly divided in five groups (n = 6): control (C) – rats not submitted to any procedure; ischemia (I) – animals submitted to ischemia-inducing procedure without pharmacologic treatment; pentoxifylline (Pen) – rats submitted to procedure and treated with pentoxifylline 3 mg/kg twice a day for 6 weeks;[8,9] cilostazol (Cil) – animals submitted to procedure and treated with cilostazol 30 mg/kg twice a day for 6 weeks, utilizing 2% gum arabic as vehicle;[6] and sham (S) – animals submitted to procedure without artery interruption. During the treatment phase, animals were not exercised on the treadmill.

Rat hindlimb ischemia model. Anesthesia was induced with intraperitoneal 10% ketamine (0.1 mL/100 g body weight) and 10% xylasine (0.05 mL/100 g body weight). Hindlimb ischemia was induced by left common iliac artery interruption, following the surgical procedure described in detail elsewhere.[9] Careful dissection was performed not to damage nerves and lymphatic vessels. No heparin or antiplatelet agents were given. In the sham group, the procedure was done without artery interruption.

Computed treadmill gait analysis. A computed treadmill developed at University of São Paulo, Department of Pharmacology of Faculty of Medicine of Ribeirão Preto was utilized for gait analysis. It consisted of a moving belt made of a mesh of stainless steel, which transmitted electric impulses. Light electrodes (0.1 g) were symmetrically placed in each animal's hindlimbs with adhesive tape. As the animals walked on the stainless steel mesh, contacts between each hindlimb and the moving surface were registered. Time, number, and duration (ms) of each contact were obtained. Data were analyzed by specially developed software. Animal's gait pattern was analyzed by plotting cumulative contacts vs time for each hindlimb. The sum of total number of contacts (TNC) and the total duration of contacts (TDC) were compared between left and right hindlimbs and among groups. Ischemic incapacitation was defined as the deficiency of the ischemic limb to contribute

to animal gait. Quantification of ischemic incapacitation was done comparing TNC and TDC between both hindlimbs. The left hindlimb ischemic incapacitation index (LHII), which was compared among groups, was defined by the formula:

$$LHII = \left(1 - \frac{TNC_{left} \times TDC_{left}}{TNC_{right} \times TDC_{right}}\right) \times 100$$

For gait analysis, animals were placed on the treadmill and walked spontaneously for 5 minutes at a speed of 12m min^{-1} and angulation of 15°.

Statistical analysis. Data were expressed as the mean ± the standard deviation. Statistical comparisons within and among groups were done with mixed effects linear regression. Statistical comparisons between animal weight values were done with Kruskal–Wallis one-way analysis of variance. A *P* value of less than .05 was considered statistically significant.

RESULTS

Weight and clinical evaluation

There was no significant difference among groups between animal preprocedure weight values and after 6 weeks. Ulcers, tissue loss, and wound infection were not observed in any animal after the procedure. In groups I, Pen, and Cil, which were subjected to iliac artery interruption, pallor was observed during the first days that turned progressively into cyanosis. This cyanosis was more evident during exercise on the treadmill.

Gait analysis

Contact vs time. A separate analysis of a random animal of ischemic and nonischemic groups for 120 seconds, plotting each contact vs time for each hindlimb generated a graph with two lines. In groups C and S, these lines were parallel, whereas in groups I, Pen, and Cil, these lines were divergent, with the line representing the left hindlimb being below. A sample graph of an animal of S group and I group is showed in Fig 1.

TNC. Comparing TNC between left and right hindlimbs, groups C and S presented no significant difference. In I, Pen, and Cil groups, left hindlimbs had significantly less contacts, evidencing a gait disturbance (Table I).

Comparing right hindlimb TNC among groups, there was no significant difference. Left hindlimb TNC values were significantly lower in I, Pen, and Cil groups than in C and S groups (*P* < .01). When compared separately, I, Pen, and Cil groups were not different (Fig 2).

TDC. In C and S groups, TDC was not different between left and right hindlimbs. In I, Pen, and Cil groups, TDC values were significantly lower for left hindlimb when compared with right hindlimb (Table II).

Left hindlimb TDC values were significantly lower in I and Pen groups than the other groups (*P* < .01). Cil group presented higher values, not significantly different form C and S groups (*P* = .16). Right hindlimb TDC values were significantly greater for I group (Fig 3).

Contact x time
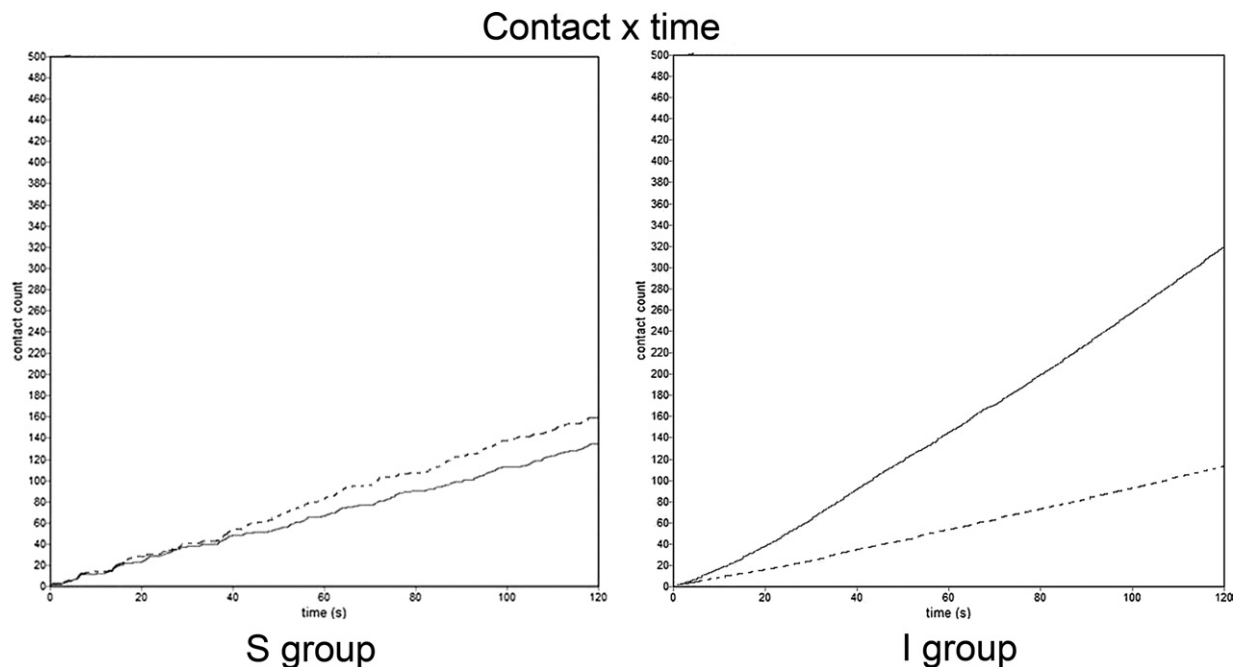
Fig 1. Plotting contact vs time for each hindlimb in sham (*S*) and ischemia and no treatment (*I*) groups. *Straight line*, right hindlimb; *interrupted line*, left hindlimb.

Table I. Intragroup comparison of TNC of each hindlimb

	TNC		
Groups	*Right hindlimb (mean ± SD)*	*Left hindlimb (mean ± SD)*	*P*
C	647.67 ± 93.43	655.50 ± 50.46	.80
I	701.00 ± 148.85	337.00 ± 97.89	<.01
Pen	623.50 ± 62.94	252.50 ± 59.88	<.01
Cil	619.33 ± 185.09	416.83 ± 99.79	<.01
S	559.33 ± 51.84	587.00 ± 65.35	.39

C, Control; *Cil,* ischemia and cilostazol; *I,* ischemia and no treatment; *S,* sham; *SD,* standard deviation; *Pen,* ischemia and pentoxifylline; *TNC,* total number of contacts.

LHII. LHII was significantly increased in I and Pen groups compared with C and S groups ($P < .01$). Cil group presented a LHII significantly lower than I and Pen groups ($P < .01$) but higher than C and S groups ($P < .1$) (Fig 4).

DISCUSSION

All knowledge on human IC is based on a biped model of locomotion, in which gait is developed in two limbs.[10] Gait disturbances in patients with chronic limb ischemia were characterized by a decrease in speed, step length, and cadence.[11] The detection of gait disturbances resembling intermittent claudication in quadruped animals implies adopting a model that takes into consideration the mechanisms of gait in four limbs.

The present study adopted the rat hindlimb ischemia model as described by Hudlincka et al[9] with addition of methods of gait analysis described by Tonussi et al.[12] These authors used a computed treadmill to determine the functional incapacitation caused by a mechanical lesion in the rat knee. Time and number of each contact were used as parameters of incapacitation. Models of hindlimb ischemia models were previously described in rats.[13-15]

In the present investigation, ischemia was induced by left common iliac artery interruption, since it permitted comparisons between ischemic and nonischemic hindlimbs. Midline abdominal incision was the access of choice in order not to interfere in gait symmetry. Rochester et al studied hemodynamic alterations caused by high left common iliac artery interruption and determined that 6 weeks was the time to achieve chronic limb ischemia.[16] Thus, this was the time chosen for the treadmill test after the procedure.

Testing medications for treatment of intermittent claudication was the main motivation of this study. In a previous animal study, authors used muscle exhaustion time for testing medications, which was defined as animal inability to continue treadmill gait.[17] Another work used the maximum distance reached by a rat in a treadmill to study the effect of a prostaglandin E_1 analogue in a model of neurologic claudication.[18]

In the present study, we used TNC and TDC as parameters of gait analysis. Left hindlimb TNC and TDC values were lower in ischemic groups in comparison with nonischemic ones ($P < .01$). Thus, the treadmill test was able to distinguish ischemic and nonischemic animals. LHII is relative quantity that correlates gait performance of the ischemic limb with the contralateral one. Studying gait and
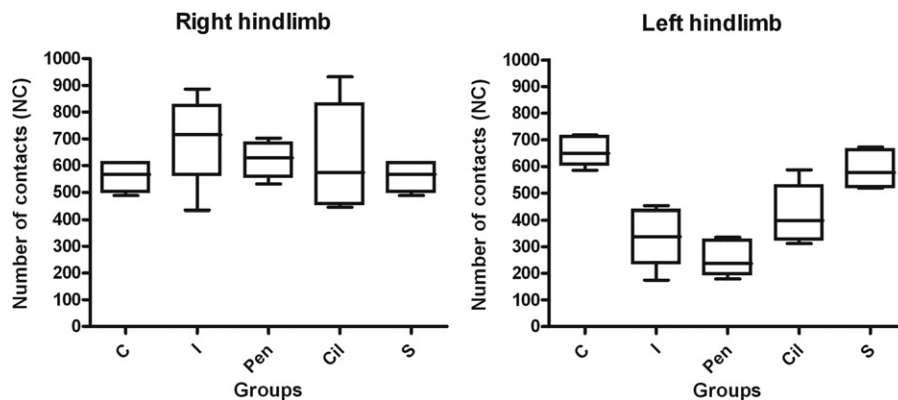
Fig 2. Intergroup comparison of total number of contacts (TNC) of each hindlimb. *C*, Control; *Cil*, ischemia and cilostazol; *I*, ischemia and no treatment; *Pen*, ischemia and pentoxifylline; *S*, sham; (—), mean; (□), confidence interval; (‡), standard deviation.

Table II. Intragroup comparison of TDC of each hindlimb

	TDC		
Groups	*Right hindlimb*	*Left hindlimb*	P
C	139.50 ± 27.37	137.33 ± 30.26	.84
I	199.17 ± 25.30	71.83 ± 24.56	<.01
Pen	153.50 ± 24.17	61.50 ± 22.41	<.01
Cil	142.83 ± 34.75	115.67 ± 23.67	.01
S	141.50 ± 20.05	137.50 ± 22.23	.71

C, Control; *Cil,* ischemia and cilostazol; *I,* ischemia and no treatment; *Pen,* ischemia and pentoxifylline; *S,* sham; *SD,* standard deviation; *TDC,* total duration of contacts.

defining percent of usage of ischemic hindlimb, one can estimate incapacitation generated by ischemia, and perform comparisons within and among groups testing different therapeutic options.

There was no weight difference among groups 6 weeks after ischemia-inducing procedure. This fact, as well as the absence of ulcers and tissue loss, supported that left common iliac artery interruption did not cause rest pain that could lead the animal to starvation and weight loss.

TNC was compared within and among groups. In nonischemic groups (C, S), TNC was the same for both limbs, demonstrating that the sham procedure did not impair the ability to walk. However, in ischemic groups (I, Pen, and Cil), left hindlimb TNC values were lower than right hindlimb ones, indicating that the system was sensitive in detecting ischemic gait disturbances.

Comparing right hindlimb TNC among groups, no difference was found. Since the rat is a tetrapod, there was no overload on hind limbs, even in the ischemic groups. These data suggest that forelimbs may contribute to this compensation.

Besides being lower in all ischemic groups, left hindlimb TNC values were equivalent in animals treated with cilostazol, pentoxifylline, and in ischemic animals without treatment.

TNC, analyzed independently, did not show improvement in gait performance in groups treated with cilostazol and pentoxifylline compared with ischemic groups.

In ischemic groups, TDC values were lower for left hindlimbs than right hindlimbs, while in nonischemic ones there was no significant difference between hindlimbs. This was additional evidence that the system was sensitive in detecting ischemic gait disturbances.

Comparison of right hindlimb TDC among groups showed that ischemic animals had greater values than nonischemic ones. This suggested that animals favored the nonischemic hindlimb (right) during deambulation. However, in the group treated with cilostazol, right hindlimb TDC values were similar to those found in nonischemic groups. This was not observed in the group treated with pentoxifylline. Left hindlimb TDC values were greater in nonischemic groups, except for the group treated with cilostazol. Left hindlimb TDC values were not significantly different between the Cil group and the nonischemic groups (P = .16). These findings demonstrated that cilostazol caused an improvement in gait performance.

By means of LHII, the global gait performance of hind limbs could be evaluated. Animals treated with cilostazol for 6 weeks had significant improvement in treadmill test. Groups treated with pentoxifylline presented performance equivalent to animals without treatment.

Orito et al also used an experimental model with treadmill tests in ischemic animals to evaluate IC.[6] Their parameters were the distance walked without disturbance and maximal walked distance. They demonstrated that cilostazol at a dose of 30 mg/kg twice a day for 2 weeks increased distance walked without gait disturbance and also maximal walked distance. At a dose of 100 mg/kg, cilostazol did not cause benefit, since it decreased systemic blood pressure.

In humans, clinical benefits of pentoxifylline are questionable. This drug promoted only a modest increase in walked distance in treadmill tests, and its use in IC lacks clinical evidence.[19] Cilostazol has a number of actions that may contribute to the amelioration of chronic limb ischemia, such
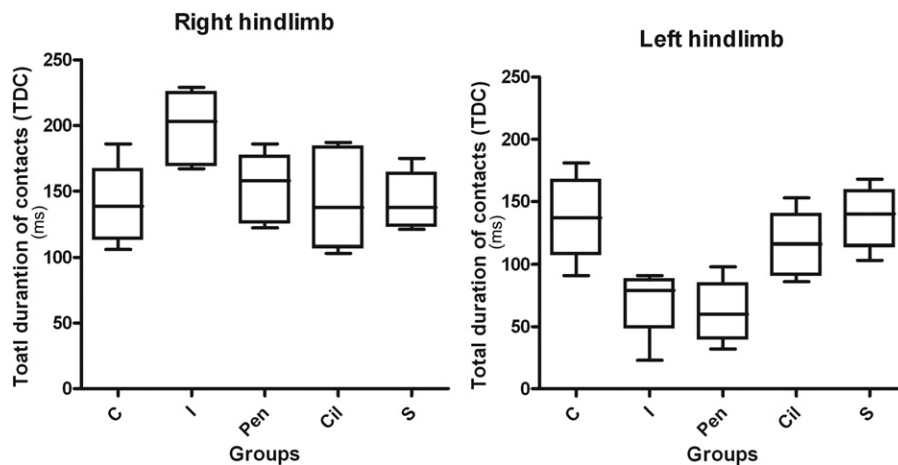
Fig 3. Intergroup comparison of total duration of contacts (TDC) of each hindlimb. *C*, Control; *Cil*, ischemia and cilostazol; *I*, ischemia and no treatment; *Pen*, ischemia and pentoxifylline; *S*, sham; (—), mean; (□), confidence interval; (‡), standard deviation.

Fig 4. Left hindlimb ischemic incapacitation index (*LHII*) in different groups. *C*, Control; *Cil*, ischemia and cilostazol; *I*, ischemia and no treatment; *Pen*, ischemia and pentoxifylline; *S*, sham; (—), mean; (□), confidence interval; (‡), standard deviation.
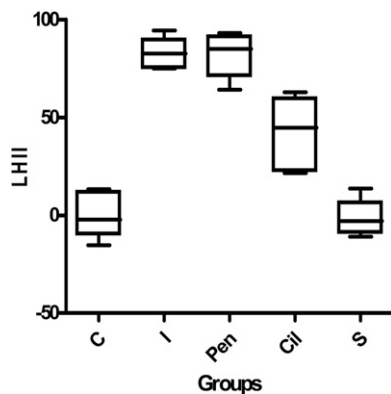
as the inhibition of platelet aggregation, which is caused by inhibiting phosphodiesterase III[20,21] and increasing blood flow during reactive hyperemia.[22,23] Strong clinical evidence supports this drug as pharmacologic treatment for patients with intermittent IC.[24] A meta-analysis of 1751 patients demonstrated clinical benefits of cilostazol. Treatment with cilostazol was associated with greater improvements in walking ability and quality of life.[25] A multicentric randomized trial comparing clinical effects of cilostazol and pentoxifylline in IC showed that cilostazol was significantly better than pentoxifylline for increasing walking distances.[26] Several other multicentric studies and meta-analyses demonstrate that cilostazol causes improvement of maximal walking distance and metabolic parameters in IC.[27-31]

The findings of this study added evidence to the available clinical data, contributing to the idea that cilostazol provides benefits in the treatment of IC.[32-35] Our study also concluded

that pentoxifylline has a limited role, and its use lacks clinical and experimental evidence.[36,37] The compatibility of the present results with the available clinical data suggests that the adopted model may be applicable for future studies. New therapies for IC, such as drugs, gene therapy, or other molecular strategies could be evaluated with this model.

There were some limitations of this work. Limb perfusion was evaluated only on a clinical basis, with observation of pallor and cyanosis. Objective measurements such as transcutaneous oxygen saturation and Doppler ultrasound were not performed. Platelet function was also not studied. Gait speed was not evaluated, but it is a promising new field of research for future studies, which could bring improvement to the model. Finally, anatomical and histologic examinations of the ischemic hindlimbs were not performed after the euthanasia.

In conclusion, cilostazol at a dose of 30 mg/kg twice a day promoted improvement in gait performance in rats submitted to chronic hindlimb ischemia. Pentoxifylline at a dose of 3 mg/kg twice a day did not show this effect.

AUTHOR CONTRIBUTIONS

Conception and design: MB, WP, CP
Analysis and interpretation: MB, WP
Data collection: MB
Writing the article: MB, MD
Critical revision of the article: CP, EJ, MD
Final approval of the article: CP, MD, EJ
Statistical analysis: MB, WP
Obtained funding: CP
Overall responsibility: MB

REFERENCES

1. Smith FB, Lowe GD, Lee AJ, Rumley A, Leng GC, Fowkes FG. Smoking, hemorheologic factors, and progression of peripheral arterial disease in patients with claudication. J Vasc Surg 1998;28:129-35.
2. Chen SJ, Pipinos I, Johanning J, Radovic M, Huisinga JM, Myers SA, et al. Bilateral claudication results in alterations in the gait biomechanics at the hip and ankle joints. J Biomech 2008;41:2506-14.

3. Beebe HG, Dawson DL, Cutler BS, Herd JA, Strandness DE Jr, Bortey EB, et al. A new pharmacological treatment for intermittent claudication: results of a randomized, multicenter trial. Arch Intern Med 1999; 159:2041-50.

4. Dawson DL, Cutler BS, Hiatt WR, Hobson RW, Martin JD, Bortey EB, et al. A comparison of cilostazol and pentoxifylline for treating intermittent claudication. Am J Med 2000;109:523-30.

5. Liu Y, Fong M, Cone J, Wang S, Yoshitake M, Kambayashi J. Inhibition of adenosine uptake and augmentation of ischemia-induced increase of interstitial adenosine by cilostazol, an agent to treat intermittent claudication. J Cardiovasc Pharmacol 2000;36:351-60.

6. Orito K, Kishi M, Fujiki H, Nakazawa T, Imaizumi T, Kimura Y, et al. A method for evaluating drug effects on intermittent claudication using a treadmill in rats with unilateral hindlimb artery occlusion. J Pharmacol Toxicol Methods 2004;49:25-9.

7. Piccinato CE, Sousa AC, Prado WA, Messias A, Bredarioli M, Dalio MB, et al. Assessment of gait dynamics in rats submitted to limb ischemia. Acta Cir Bras 2011;26:490-5.

8. Hudlická O, Price S. Effects of torbafylline, pentoxifylline and buflomedil on vascularisation and fibre type of rat skeletal muscles subjected to limited blood supply. Br J Pharmacol 1990;99:786-90.

9. Hudlicka O, Price S. The role of blood flow and/or muscle hypoxia in capillary growth in chronically stimulated fast muscles. Pflugers Arch 1990;417:67-72.

10. Kruidenier LM, Nicolaï SP, Ten Bosch JA, De Bie RA, Prins MH, Teijink JA. Predictors of walking distance after supervised exercise therapy in patients with intermittent claudication. Eur J Vasc Endovasc Surg 2009;38:449-55.

11. Scherer SA, Bainbridge JS, Hiatt WR, Regensteiner JG. Gait characteristics of patients with claudication. Arch Phys Med Rehabil 1998;79: 529-31.

12. Tonussi CR, Ferreira SH. Rat knee-joint carrageenin incapacitation test: an objective screen for central and peripheral analgesics. Pain 1992;48:421-7.

13. Nicholson CD. Experimental models of chronic lower extremity arterial occlusive disease: lessons for drug development. Vasc Med 1996;1: 43-9.

14. Yang HT, Ogilvie RW, Terjung RL. Peripheral adaptations in trained aged rats with femoral artery stenosis. Circ Res 1994;74:235-43.

15. Yoshida M, Horimoto H, Mieno S, Nomura Y, Okawa H, Nakahara K, et al. Intra-arterial bone marrow cell transplantation induces angiogenesis in rat hindlimb ischemia. Eur Surg Res 2003;35:86-91.

16. Rochester JR, Brown NJ, Reed MW. Characterisation of an experimental model of chronic lower limb ischaemia in the anaesthetised rat. Int J Microcirc Clin Exp 1994;14:27-33.

17. Tiidus PM, Bombardier E. Oestrogen attenuates post-exercise myeloperoxidase activity in skeletal muscle of male rats. Acta Physiol Scand 1999;166:85-90.

18. Nakai K, Takenobu Y, Takimizu H, Akimaru S, Maegawa H, Ito H, et al. Effects of OP-1206 alpha-CD on walking dysfunction in the rat neuropathic intermittent claudication model: comparison with nifedipine, ticlopidine and cilostazol. Prostaglandins Other Lipid Mediat 2003;71:253-63.

19. Olin JW, Sealove BA. Peripheral artery disease: current insight into the disease and its diagnosis and management. Mayo Clin Proc 2010;85: 678-92.

20. De Backer T, Vander Stichele R, De Buyzere M, De Backer G, Van Bortel L. Silence of the limbs pharmacological symptomatic treatment of intermittent claudication. Curr Vasc Pharmacol 2010;8:383-7.

21. Sallustio F, Rotondo F, Di Legge S, Stanzione P. Cilostazol in the management of atherosclerosis. Curr Vasc Pharmacol 2010;8:363-72.

22. Pande RL, Hiatt WR, Zhang P, Hittel N, Creager MA, McDermott M. A pooled analysis of the durability and predictors of treatment response of cilostazol in patients with intermittent claudication. Vasc Med 2010;15:181-8.

23. Rizzo M, Corrado E, Patti AM, Rini GB, Mikhailidis DP. Cilostazol and atherogenic dyslipidemia: a clinically relevant effect? Expert Opin Pharmacother 2011;12:647-55.

24. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, et al. Inter-Society Consensus for the management of peripheral arterial disease (TASC II). J Vasc Surg 2007;45(Suppl S):S5-67.

25. Regensteiner JG, Ware JE, Jr, McCarthy WJ, Zhang P, Forbes WP, Heckman J, et al. Effect of cilostazol on treadmill walking, community-based walking ability, and health-related quality of life in patients with intermittent claudication due to peripheral arterial disease: meta-analysis of six randomized controlled trials. J Am Geriatr Soc 2002;50:1939-46.

26. Dawson DL, Zheng Q, Worthy SA, Charles B, Bradley DV Jr. Failure of pentoxifylline or cilostazol to improve blood and plasma viscosity, fibrinogen, and erythrocyte deformability in claudication. Angiology 2002;53:509-20.

27. Accetto B. Beneficial hemorheologic therapy of chronic peripheral arterial disorders with pentoxifylline: results of double-blind study versus vasodilator-nylidrin. Am Heart J 1982;103:864-9.

28. Elam MB, Heckman J, Crouse JR, Hunninghake DB, Herd JA, Davidson M, et al. Effect of the novel antiplatelet agent cilostazol on plasma lipoproteins in patients with intermittent claudication. Arterioscler Thromb Vasc Biol 1998;18:1942-7.

29. Mohler ER, III, Beebe HG, Salles-Cuhna S, Zimet R, Zhang P, Heckman J, et al. Effects of cilostazol on resting ankle pressures and exercise-induced ischemia in patients with intermittent claudication. Vasc Med 2001;6:151-6.

30. Money SR, Herd JA, Isaacsohn JL, Davidson M, Cutler B, Heckman J, et al. Effect of cilostazol on walking distances in patients with intermittent claudication caused by peripheral vascular disease. J Vasc Surg 1998;27:267-74.

31. Thompson PD, Zimet R, Forbes WP, Zhang P. Meta-analysis of results from eight randomized, placebo-controlled trials on the effect of cilostazol on patients with intermittent claudication. Am J Cardiol 2002;90:1314-9.

32. De Backer TL, Vander Stichele RH, Warie HH, Bogaert MG. Oral vasoactive medication in intermittent claudication: utile or futile? Eur J Clin Pharmacol 2000;56:199-206.

33. Ikewaki K, Mochizuki K, Iwasaki M, Nishide R, Mochizuki S, Tada N. Cilostazol, a potent phosphodiesterase type III inhibitor, selectively increases antiatherogenic high-density lipoprotein subclass LpA-I and improves postprandial lipemia in patients with type 2 diabetes mellitus. Metabolism 2002;51:1348-54.

34. Ito C, Kusano E, Furukawa Y, Yamamoto H, Takeda S, Akimoto T, et al. Modulation of the erythropoietin-induced proliferative pathway by cAMP in vascular smooth muscle cells. Am J Physiol Cell Physiol 2002;283:C1715-21.

35. Pipinos II, Sharov VG, Shepard AD, Anagnostopoulos PV, Katsamouris A, Todor A, et al. Abnormal mitochondrial respiration in skeletal muscle in patients with peripheral arterial disease. J Vasc Surg 2003;38: 827-32.

36. Müller R. Pentoxifylline–a biomedical profile. J Med 1979;10:307-29.

37. Porter JM, Cutler BS, Lee BY, Reich T, Reichle FA, Scogin JT, et al. Pentoxifylline efficacy in the treatment of intermittent claudication: multicenter controlled double-blind trial with objective assessment of chronic occlusive arterial disease patients. Am Heart J 1982;104: 66-72.