Contribution to prevention of cerebral ischemia by cilostazol, a phosphodiesterase III inhibitor: a review

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ABSTRACT

This literature review aims at presenting the main possibilities for the clinical application of cilostazol in the central nervous system. Cilostazol, a selective phosphodiesterase type III inhibitor, increases adenosine 3′,5′-cyclic monophosphate levels on platelets, endothelial and smooth muscle cells, having vasodilatory, antiplatelet and antithrombotic properties. Currently, it is the first-choice drug for intermittent claudication, due to peripheral occlusive vascular disease. In addition, there is evidence showing that cilostazol is efficacious in cerebral atherosclerotic process, resulting in increase of blood flow and volume and preventing infarctions, especially lacunar and recurrent, since it reduces cellular death due to apoptosis and oxidative stress in white and gray substances.

Keywords: Phosphodiesterase inhibitors, cerebral infarction, cerebral blood flow, cilostazol.

RESUMO

Esta revisão bibliográfica objetiva expor estas pesquisas sobre as ações do cilostazol no sistema nervoso central. O cilostazol é uma droga que demonstrou exercer inibição seletiva e potente da fosfodiesterase tipo III, ocasionando o aumento de adenosina cíclica -3′,5′-monofosfato nas plaquetas, nas células endoteliais e nas células musculares lisas, sendo classificado como vasodilatador, antiagregante plaquetário e antitrombótico. É o fármaco de primeira escolha na
Introduction

Reduced cerebral blood flow implies not only vascular dementia caused by multiple infarctions, but also other types of mental deterioration, including Alzheimer's disease. White matter lesions, such as hypertension and cerebrovascular changes, are more common in old age, being responsible for cognitive decline and walking disorders. Loss of myelin an axonal damage were identified in experimental chronic cerebral ischemia, induced by permanent occlusion of the common carotid artery in rats, whose model has been proposed for vascular dementia and circulatory lesions in the white matter. Suppression of microglia, which is activated by contact with neural damage, and mitigation of white matter lesions using an antiinflammatory drug (nimesulide) have been demonstrated in a previous study, suggesting the importance of inflammatory reaction in causing lesions in that brain region. Microglia, playing a major role in development and remodeling of the central nervous system (CNS), followed by astrocytes, which form the glia and contribute to the blood-brain barrier, are the greatest sources of tumor necrosis factor alpha (TNF-α). TNF-α, as a proinflammatory cytokine, induces formation of post-ischemia and loss of myelin. On the other hand, death of oligodendroglial cells was reported as occurring after bilateral carotid occlusion in gerbils and in transient global ischemia in rats. Previous research studies demonstrated that the optic tract, submitted to chronic cerebral hypoperfusion, caused activation of microglial cells, exacerbating white matter lesions and producing reactive oxygen species, besides proinflammatory cytokines, such as TNF-α.

There is accumulated evidence that oligodendroglial apoptosis in human brains is increased in white matter ischemic lesions, consisting of myelin degeneration, astrogliosis, microglia activation and loss of oligodendroglia. Oligodendroglia are cells that form myelin sheaths in brain axons and are more likely to have oxidative stress than other glial cells, such as astrocytes, causing apoptosis with activation of caspases, enzymes that are proapoptotic markers.

Also, cells with in situ nick end labeling (TUNEL), a method to detect areas of deoxyribonucleic acid (DNA) that are eliminated during apoptosis, and cytochrome c, which is an initial mediator of caspase activation, secreted by mitochondria during hypotonic lysis of cells, are manifested ischemia. Chronic cerebral hypoperfusion causes increase in glial fibrillary acidic protein (GFAP), a marker for astrocytes that is the intermediate filament only found in glial cells or that have glial origin, and in anti-CD11b monoclonal antibody (OX-42), a marker of microglia in the optic tract, indicating microglia hyperactivation, whereas 3',5'-cyclic nucleotide 3'-phosphodiesterase (CNPase), a marker of oligodendrocytes, is significantly reduced, suggesting increase in their death.

Among the factors responsible for apoptosis is the family of B-cell-leukemia/lymphoma-2 (Bcl-2)
genes. The Bcl-2 gene has been suggested as suppressor of cell death due to apoptosis in a variety of in vitro systems and in cell lineages (reed). The X protein associated with Bcl (BAX) is a member of the Bcl-2 family that, on the contrary, causes cell death due to mitochondrial dysfunction and caspase activation.

The phosphatidylinositol 3-kinase/serine-threonine protein (PI3K/Akt) metabolic pathway plays a major role to regulate several cell functions, including growth, differentiation and apoptosis. In addition, apoptosis contains protein kinase 2 (CK2), which is a transcription factor present in nucleus and cytoplasm that is regulated by calcium. It is involved in many metabolic processes related to cell growth and proliferation, catalyzed by phosphorilation of a large number of protein kinase. On the other hand, there is phosphorilation of the phosphatase gene and tensin homologue deleted on chromosome 10 (PTEN), apoptosis controller through cell regulation and viability by caspase activation and inhibition of DNA-binding protein to cyclic adenosine 3',5'-monophosphate (CREB), a cascade activated by multiple growth factors and cytokines that regulate cell survival and growth. CREB fosters cell survival, including neurons.

**Cilostazol pharmacology**

Cilostazol is indicated for clinical treatment of intermittent claudication caused by peripheral occlusive arterial disease (POAD), a situation in which the results of two meta-analyses demonstrated superiority in relation to placebo and pentoxifylline, since it increases painless walking distance and functional capacity, bringing benefits to quality of life. In that situation, according to the guidelines of the American College of Cardiology and the American Heart Association, its recommendation degree is class I (procedure is useful and effective) and strength of scientific evidence A (based on multiple randomized studies).

This drug is classified as platelet antiaggregating and anti-thrombotic agent with vasodilating action, and there is no evidence of prolonged bleeding time when compared with aspirin, clopidogrel or ticlopidine, even using a combination of those (Comerota). It causes potent and selective phosphodiesterase III inhibition, causing increase in cAMP because it blocks hydrolysis in thrombocytes and smooth muscle cells, reducing intracellular calcium with consequent relaxation and vasodilation. cAMP, in turn, is one of the regulators of inflammatory and immunologic reactions. Cilostazol, therefore, is directly associated with the opening of high-conductance calcium-activated potassium channels (Maxi-K) via activation of kinase proteins and also TNF-α suppression and expression of adherent cells.

A preclinical study demonstrated that cilostazol resulted in inhibition of thrombosis formation in the carotid artery, and that it could be useful to prevent strokes. In a study of placebo-controlled cilostazol including 141 patients with type II diabetes mellitus, followed for 12 months, there was reduction in carotid intimal-medial thickness verified by high-resolution B-mode ultrasound (p < 0.05, even after correction for risk factors, such as hypertension, smoking and dyslipidemia), which could have beneficial effect in atherosclerosis. Another study with similar design obtained a similar result in 62 patients followed for 2.6 years (p < 0.05). Its efficacy as antiplatelet therapy to prevent cerebral infarction has also been demonstrated.

The most frequent side effects include headache, tachycardia, palpitations, loose stools and diarrhea. In two studies there was need of interrupting use of cilostazol due to headache in 1.7% of patients, in relation to 1.3% in those treated with placebo, whereas suspension due to other causes was similar between groups. In hepatic insufficiency, Child-Pugh classes B and C, prescription should be careful. It is contraindicated in patients with congestive heart failure or
ejection fraction of the left ventricle lower than 40%.42

Actions of cilostazol in central nervous system blood flow

Lee et al. demonstrated regression of cerebral lesion size induced by focal cerebral ischemia through edema reduction and improvement in neurologic deterioration in rats treated with cilostazol43 and improvement in spatial memory learning (p < 0.05).44 There was also reduction in white and gray matter cerebral lesion volume in rodents submitted to focal cerebral ischemia in 45% (p < 0.02) and axonal damage in 42.4% (p < 0.002) compared with placebo, and increase in cerebral blood flow and volume in the peri-infarction region (p < 0.05).45

This drug caused dilatation of the medial cerebral artery in pigs, even with possible nitric oxide disorder resulting from cerebral infarction and vasospasm,46 a result that was also found in human beings, but with no increase in regional flow (p = 0.02).47

Cilostazol had dose-dependent vasodilation in penetrating cerebral arterioles in rabbits, pressurized in vitro independently of nitric oxide synthase antagonism by LG-nitroarginine methyl ester, and inhibition of vasodilating prostaglandins by aspirin or by endothelial cell chemical denudation. The same occurred in the presence of thromboxane A2 and 5-hydroxytryptamine, when it seems to have direct action on vessel resistance-diameter binomial to change blood flow rate, and may contribute to prevention of cerebral lacunar infarction,48 a disease defined as a small infarction involving occlusion of penetrating arterioles.49 In cats, it inhibits formation of platelet thrombi after medial cerebral artery occlusion and induces significant dilatation.50

In another study, there was suppression of astrocyte and microglial cell activation, besides increase in number of oligodendrocytes, secondary to chronic cerebral hypoperfusion, reducing cell death due to apoptosis, in association with reduced production of TNF-α, caspase-3-positive cells, TUNEL-positive cells and OX-42 (p < 0.05), GFAP and CNPase (p < 0.01), in the white matter of rats submitted to bilateral occlusion of common carotid arteries, preventing formation of vacuoles and rarefaction.51

Cilostazol caused reduction in BAX and cytochrome c, as well as increase in Bcl-2, ensuring survival after cerebral ischemia in rats,52 whose ability may be associated with its capacity of maintaining the calcium rate and increasing the membrane potential of mitochondria. It showed neuroprotective effect against focal cerebral ischemia associated with antiapoptotic activity, reducing TNF-α, BAX, cytochrome c, and increasing Bcl-2 (p < 0.001), suppressing DNA fragmentation and cell death due to oxidative stress in animals submitted to medial cerebral artery occlusion.53

It reduced TNF-α level and PTEN phosphorylation, increasing Akt and CREB phosphorylation in culture of human cortical neurons (HCN 1A); in addition, it increased CK2 phosphorylation in human neuroblastoma cells (SK-N-SH), opening maxi-K channels54 and corroborating previous findings.55 Cilostazol also reduced PTEN phosphorylation and increased the level of CK2 (p < 0.001), Akt and CREB phosphorylation, in association with increase in Bcl-2 protein in the ischemia area, besides opening maxi-K channels in rats.56 Such cilostazol activity can be due to its function in opening of calcium channels, associated with regulation of homeostase in mitochondria, which is essential for cell survival,57 avoiding white matter apoptosis.58 It reduced apoptosis by reducing DNA fragmentation, CREB and PTEN phosphorylation rate, causing regression in size of cerebral infarction in animals submitted to focal cerebral ischemia.59
The TOSS study,\textsuperscript{60} a randomization of 135 patients with acute symptomatic stenosis of the medial or basilar cerebral artery after stroke followed by 6 months, showed progression of the atherosclerotic plaque in 6.7% in the cilostazol group vs. 28.8% in the control group (p = 0.008), and a plaque regression in 24.4% in the cilostazol group vs. 15.4% in the control group.

In a multicenter clinical trial including 1,052 placebo-controlled cilostazol patients, it was demonstrated that a 12-month administration was safe and effective in preventing recurrent cerebral infarction, with a 41.7% reduction in relative risk (p = 0.015; CI95% 9.2-62.5), especially lacunar infarction, without affecting occurrence of intracranial hemorrhage,\textsuperscript{61} stressing the drug neuroprotective vascular activity\textsuperscript{62} and signaling a possible beneficial cost-effectiveness ratio in pharmacoeconomic analysis, when compared to aspirin.\textsuperscript{63} Prevention of silent cerebral infarction was reported in a study of 89 patients, placebo-controlled, and with a 3.6-year follow-up (p < 0.001). Mochizuki et al., using single photon emission computed tomography (SPECT),\textsuperscript{64} demonstrated increased cerebral blood flow in a non-controlled study with a 3-month follow-up. Oishi et al. reported increased blood flow in the frontal white matter, temporal and occipital cortex in relation to ticlopidine in patients in chronic stage of cerebral infarction, also using SPECT.\textsuperscript{65} There was also evidence of increase in cerebral flow in patients with chronic cerebral infarction after administration for 2 weeks both in the affected and non-affected side, using the method of xenon-133 inhalation.\textsuperscript{66}

**Conclusion**

Use of cilostazol is well established in POAD, and may play a major role in cerebral atherosclerosis, both as vasodilator\textsuperscript{28} and to stabilize calcium rate, increasing the membrane potential of mitochondria.\textsuperscript{56}

Therefore, it has demonstrated its activity in microglia and astrocyte suppression and stimulation of oligodendrocytes. It also reduced cell death due to apoptosis and oxidative stress, with activity in proinflammatory cytokines, DNA fragmentation,\textsuperscript{51} besides reducing size of ischemic lesions in experimental studies.\textsuperscript{48}

In clinical research, cilostazol proved to be effective in increasing cerebral blood flow,\textsuperscript{64-66} in reducing relative risk of recurrent cerebral infarction, especially lacunar, and was useful in secondary prevention.\textsuperscript{61}

**References**


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