Dipyridamole plus Triflusal versus Triflusal Alone in Infarct Reduction after Middle Cerebral Artery Occlusion

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Background and Purpose: The objective of this work is to study the dose-dependent effect of combination therapy with dipyridamole and triflusal over that of triflusal alone on infarct size after middle cerebral artery occlusion (MCAO) ischemia. *Materials and Methods:* Male Wistar rats were subjected to a permanent MCAO in the right hemisphere. Rats received triflusal alone and with dipyridamole via oral route. Three days after surgery, infarct volumes were measured. *Results:* The lower dose regime of triflusal (10 mg/kg) and dipyridamole (200 mg/kg) caused the greatest decrease in infarct size compared with higher dose regime of triflusal (30 mg/kg) and dipyridamole (200 mg/kg) ($P < .01$), triflusal (30 mg/kg) alone ($P < .07$), and vehicle-treated controls. *Conclusions:* The lower dose combination of dipyridamole and triflusal appears to be more effective than triflusal alone after MCAO-induced cerebral ischemia. Therefore, there is a strong rationale to continue to examine the protective effects of triflusal and dipyridamole after cerebral ischemia. **Key Words:** Ischemia—MCAO—dipyridamole—triflusal—infarct.
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Introduction

Epidemiologically, neural stroke remains the most frequent cause of disability and death in industrialized and developing countries, after cancer and heart disease. Stroke is also the reason why the majority of patients suffer and are hospitalized from acute brain syndromes in the United States.[1] There are 2 major types of stroke pathogenesis: transitory or permanent occlusion of cerebral blood vessels or ischemic (more than 80% of strokes are ischemic) and hemorrhagic.[2] Both types result in subsequent tissue loss with sustainable behavioral deficits.[2-4] The middle cerebral artery (MCA) is the most universally affected blood vessel in acute ischemic disorders[5] and the most widely coagulated or occluded blood vessel.[6] By inserting an intraluminal suture[7] in the experimental rodent focal ischemia model, MCA occlusion (MCAO) produces a well-defined region of injury that includes the neocortex and the lateral striatum, resulting in underlying functional deficits.[8]

These facts make it critical to develop primary and secondary strategies to prevent or treat stroke. There are 2 major therapeutic approaches aimed at preventing or intervening after ischemia: reperfusion therapy and antiplatelet therapy. Reperfusion therapy restores blood flow through blocked arteries after stroke either by clot-busting (thrombolytic) drugs, by opening arteries with stents, or by grafting arteries around blockages. Tissue plasminogen activator efficiently improves clinical outcomes in reperfusion therapy after cerebral ischemia. However, in spite of its high efficacy, tissue plasminogen activator needs to be used within hours

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after the ischemia onset[9] with a related risk of hemorrhagic transformation.[10] Antiplatelet therapy decreases platelet aggregation and inhibits thrombus formation. Antiplatelet therapy, in addition to lifestyle changes, is the basis of arterial thrombotic stroke prevention in the secondary setting. Antiplatelet therapy also improves cardiovascular risk. Even though acetylsalicylic acid has proved its effectiveness in preventing atherothrombotic episodes, there are key limitations of this drug including the high risk of hemorrhage, limited efficacy, significant variability in interindividual response, and extended length of action that cannot be reversed if emergency surgery or hemostasis is needed.[11]

Unfortunately, newer antiplatelet therapies do not offer considerably improved protection over and above acetylsalicylic acid to treat atherothrombotic stroke. Thus, there is a need to identify and formulate new antiplatelet therapies that are appropriate for use in a larger population of stroke victims, either alone or in combination with reperfusion treatments.[12] Triflusal (2-acetyloxy-4-trifluoromethyl benzoic acid), a 4-fluoromethyl member of the salicylate family, directly inhibits cyclooxygenase-2 and irreversibly and selectively inhibits cyclooxygenase-1 and arachidonic acid metabolism in platelets. Triflusal is an indirect inhibitor of nuclear factor κB[13] and inhibits platelet aggregation, thus represents a promising substitute for acetylsalicylic acid, while presenting a more favorable safety profile. Triflusal at a dose of 30 mg/kg has been demonstrated to be effective in reducing infarct size in the rat MCAO stroke model.[14] Dipyridamole (2-{[9-(bis(2-hydroxyethyl)amino)-2,7-bis(l-piperidyl)-3,5,8,10-tetrazabicyclo [4.4.0]deca-2,4,7,9,11-pentaen-4-yl]-(2-hydroxyethyl)amino}ethanol) was introduced into clinical practice in the early 1960s as a cardioprotective coronary vasodilator.[15] Dipyridamole has both antiplatelet and vasodilatory properties with a mechanism of action that is probably related to the inhibition of platelet phosphodiesterase, stimulation of prostacyclin release, or inhibition of adenosine uptake.[16] Dipyridamole has been shown to be protective against cognitive impairment after global ischemia.[17]

The lone use of triflusal or a combined regimen of dipyridamole and triflusal treatment is more effective in the secondary prevention of atherothrombotic stroke than acetylsalicylic acid and dipyridamole combination.[18] We therefore hypothesize that the combination of suboptimal doses of dipyridamole and triflusal will provide neuroprotection by decreasing infarct size after MCAO compared with triflusal alone. At present, we aim to investigate whether acute poststroke use of dipyridamole and triflusal, which is very similar to a clinical experimental paradigm for secondary stroke prevention, offers any improvement to ischemic cerebral injury.

Materials and Methods

Animal, Treatment, and Tissue Preparation

All animal protocols were carried out according to the guidelines of the Animal Use and Care Committee of Western University (approval ID: 2008-113). Male Wistar rats (265-36 g) were anesthetized using a single intraperitoneal dose of sodium pentobarbital (60 mg/kg). The animals were positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the incisor bar below the interaural line, set at 3.3 mm. Body temperatures were maintained at 37°C. Before and after surgery, rats were housed in single cages (12/12-hour light/dark cycle) and were fed ad libitum. To model ischemic stroke, the right MCA was exposed and permanently occluded at 2 points, 1 above and 1 below the inferior cerebral vein, using a Helica thermal coagulator (a combination of helium gas and a low alternating current of 50 W). The removal of a portion of bone and the exposure and ligation of the MCA were done with the aid of a surgical operating microscope (stereomicroscope; Leica, MZ6, Wetzlar, Germany). After suturing the wound, all rats received a subcutaneous injection of 30 μg/kg buprenorphine and an intramuscular injection of 20 μL (50 mg/mL stock) of enrofloxacin antibiotic (Baytril; Bayer Inc., Toronto, ON, Canada). The rat model of MCAO is well established in our laboratory. There were 4 groups of animals (n = 8 for each group). Immediately after surgery and for 2 additional days, rats received one of the following treatment suspensions via oral gavage: 200 mg/kg dipyridamole plus 10 mg/kg triflusal (D + T10 group), 200 mg/kg dipyridamole plus 30 mg/kg triflusal (D + T30 group), or 30 mg/kg triflusal (T30 group). The control rats (control group) went through the identical steps but only received the vehicle (analytical-grade ethanol) via oral gavage. Three days after surgery, rats were euthanized with an overdose of sodium pentobarbital (160 mg/kg, intraperitoneal) and transaortically perfused, first with heparinized phosphate-buffered saline followed by a slow perfusion for about 1 hour with 2% solution of 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich, St. Louis, MO)[18] and then followed by 4% formaldehyde (pH 7.4). The brains were immediately removed and sliced 1.0 mm apart, with the help of a slicer matrix. At the time of surgery, gross cerebral hemorrhage was noticed in 3 rats; these rats were replaced at a later date to maintain the same number in each group. No mortality occurred.

Analyses

Anterior images of the stained brain slices were captured using a Nikon Digital Camera (COOLPIX P80 Tokyo, Japan) with 10.1 megapixel and 18× optical zoom, powered by a large 1/1.8-inch format, high-resolution liquid-crystal display (LCD). Serial brain sections were examined, and the infarcted tissue areas were measured and

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