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PGD_2 DP1 RECEPTOR STIMULATION FOLLOWING STROKE AMELIORATES CEREBRAL BLOOD FLOW AND OUTCOMES

A. S. AHMAD *

Department of Anesthesiology, and Center for Translational Research in Neurodegenerative Disease, University of Florida College of Medicine, PO Box 100159, Gainesville, FL, USA

Abstract—Stroke is a leading cause of death and morbidity worldwide, yet effective treatments are lacking. The association of prostaglandin D_2 and its DP1 receptor with vasculature and blood propelled us to examine whether the clinically tested DP1 receptor agonist BW245C had beneficial effects following stroke. To determine if BW245C affects basal cerebral blood flow (CBF), C57BL/6 WT and $DP1^{-/-}$ mice were given a single i.p. injection of vehicle or BW245C, and CBF was recorded for 2 h. To test the effect of BW245C on stroke, WT and $DP1^{-/-}$ mice were subjected to middle cerebral artery occlusion followed by a single i.p. injection of vehicle or 0.02, 0.2, or 2.0-mg/kg BW245C immediately before reperfusion. Functional and anatomical outcomes were determined at 96 h. We also determined the effect of BW245C on CBF in peri-infarct and core during occlusion and reperfusion. Furthermore, we tested the effect of BW245C on bleeding time and ex vivo coagulation. BW245C treatment increased the basal CBF significantly in WT but not in $DP1^{-/-}$ mice. The BW245C treatment also significantly improved functional outcome and lowered infarction volume. The multisite CBF monitoring by laser-Doppler flowmetry shows that BW245C significantly increased the CBF in peri-infarct, with a significant inverse correlation between infarction and CBF. The significantly higher infarction volume in $DP1^{-/-}$ mice remained unchanged with BW245C treatment. Moreover, BW245C preserves hemostasis in non-stroke conditions. Combined, these data suggest that the DP1 receptor is an endogenous target that can rescue the brain following stroke by regulating CBF and hemostasis.

Key words: BW245C, cerebral blood flow, cerebral ischemia, hemostasis, prostaglandin D_2.

INTRODUCTION

Every year, a substantial amount of the population endures new or recurrent stroke, yet effective therapies with wide beneficial outcomes are lacking. Decades after being identified as ''the best therapy'' against stroke, tissue plasminogen activator (tPA) still remains the only evidence-based and FDA-approved therapy (Zivin et al., 1985; The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995). However, tPA treatment is extensively time dependent, which restricts its beneficial effects to only a small percentage of the population (Kohrmann et al., 2010). The severe blood flow deficit in the ischemic core leads to an irreversible cascade of cell death within minutes, whereas the cells in the peri-infarct undergo milder insults due to having residual perfusion from collateral circulation (Liebeskind, 2003; Shuaib et al., 2011; Riva et al., 2012). Therefore, one of the objectives in rescuing the brain after stroke is to use a therapeutic intervention that augments perfusion in the penumbra (Shih et al., 2009; Sutherland et al., 2012). Although numerous trials are underway for stroke treatment, the need to find a better and effective strategy to rescue the brain after stroke still continues (Fisher et al., 2009; Eltzschig and Eckle, 2011; Sutherland et al., 2012).

Prostaglandin D_2 (PGD_2) is the most abundant prostaglandin in the brain and it exerts its actions mainly through its DP1 receptors, although DP2 receptors are also reported (Matsuoka et al., 2000). Cloning and characterization of the DP1 receptor in 1995 provided an abundant avenue to study the role of PGD_2 in various physiologic conditions (Boie et al., 1995; Thornhill and Asselin, 1999; Urade and Hayaishi, 1999). The characterization and availability of highly selective DP1 ligands such as BW245C have played a crucial role in determining the effect of this receptor in various disease conditions (Kiriyama et al., 1997; Koch et al., 2005). This receptor has been shown to protect rat hippocampal slice cultures against *N*-methyl-D-aspartate (NMDA) and oxygen-glucose deprivation-induced toxicity (Liang et al., 2005). Similarly, in a mouse neonatal hypoxic-ischemic model, DP1 receptor activation by BW245C has a neuroprotective effect (Taniguchi et al., 2007). Data from our lab show that $DP1^{-/-}$ mice have greater brain infarction compared with the WT mice. Moreover, intracerebroventricular pre-treatment with the DP1 agonist BW245C minimizes excitotoxic and ischemic brain damage (Saleem et al., 2007; Ahmad et al., 2010). *In vivo* data suggest that DP1 activation improves hemodynamics (Koch et al.,

*Tel: +1-352-294-5107; fax: +1-352-294-5060.
E-mail address: aahmad@anest.ufl.edu
Abbreviations: CBF, cerebral blood flow; LDF, laser-Doppler flowmetry; MCA, middle cerebral artery; MCAO, middle cerebral arterial occlusion; NMDA, *N*-methyl-D-aspartate; PGD_2, prostaglandin D_2; tPA, tissue plasminogen activator; TTC, 2,3,5-triphenyl-tetrazolium chloride.

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2005), which may have a beneficial effect on stroke outcomes. Moreover, it has also been reported that PGD_2 and BW245C attenuate the platelet aggregation mediated by arachidonic acid and other platelet aggregators (Whittle et al., 1983; Schuligoi et al., 2007). Although the neuroprotective effect of the DP1 receptor has been recognized recently, the therapeutic potential of DP1 activation by the systemic administration of selective pharmacologic agents after stroke has not yet been tested.

A better collateral circulation and augmented cerebral blood flow (CBF) along the ischemic/hypoxic territory have shown to have improved functional and anatomical outcomes following stroke (Liebeskind, 2003; Shuaib et al., 2011; Riva et al., 2012). Therefore, given the role of BW245C on vasodilation and the fact that this compound has already been tested in humans (Whittle et al., 1983; Shah et al., 1984), in this study, we wanted to determine whether the systemic treatment of mice with this DP1 agonist after stroke would have a beneficial effect, and whether this beneficial effect was through improved CBF and hemostasis. This study provides insight into a potential new role for BW245C in stroke, which makes the DP1 receptor a novel target that can be used in designing a better therapy to rescue the brain after stroke.

EXPERIMENTAL PROCEDURES

Mice and drugs

Adult male (4–5 months old) C57BL/6 WT and $DP1^{-/-}$ mice were used following the NIH institutional guidelines for the use and care of laboratory animals. The protocol was approved by the Johns Hopkins Medical Institutions and the University of Florida Institutional Animal Care and Use Committee. The $DP1^{-/-}$ mice were inbred and backcrossed with C57BL/6 WT (Jackson Laboratories, Bar Harbor, ME) eight to ten generations. The DP1 agonist BW245C was purchased from Cayman Chemicals, whereas the $DP1^{-/-}$ mice provided by Dr. Shuh Narumiya (Matsuoka et al., 2000) are now being bred and maintained in our breeding facility at the University of Florida. All animals were randomized and investigators responsible for surgical procedures, drug treatments, and functional and anatomical outcome measurements were blinded.

Basal CBF measurement

Mice were anesthetized with isoflurane (3.0% for induction and 1.5% for maintenance). A vertical skin incision was made between the right eye and ear, and the temporal bone was exposed by moving the temporal muscle. A fiber optic probe was placed and fixed with medical grade adhesive on the skull over middle cerebral artery (MCA) approximately 6 mm lateral and 1 mm posterior to the bregma, and CBF was monitored by laser-Doppler flowmetry (LDF) (Moor Instruments, Devon, UK). Once a stable baseline was obtained, mice (n = 6/group) were given single i.p. injections of vehicle, or 0.02-, 0.2-, or 2.0-mg/kg BW245C, and CBF was continuously recorded until 2 h after the injection. To determine the selectivity of BW245C toward the DP1 receptor and any compensatory mechanism associated with DP1 receptor deletion, the effect of 0.2-mg/kg BW245C on CBF was then tested in $DP1^{-/-}$ mice (n = 6).

MCA occlusion (MCAO) and BW245C treatment

Transient focal cerebral ischemia was induced by occluding the MCA with an intraluminal filament as described earlier (Longa et al., 1989; Ahmad et al., 2006). Briefly, WT and $DP1^{-/-}$ mice were anesthetized with continuous isoflurane anesthesia. Relative CBF over the parietal cortex was monitored by LDF by placing the probe 1 mm posterior to the bregma and 6 mm lateral to the midline. Occlusion of the MCA was accomplished by inserting a 7-0 Ethilon nylon monofilament (Ethicon, Somerville, NJ, USA) coated with flexible silicone. Successful occlusion was confirmed by a decrease of > 80% in CBF. During the 60-min occlusion period, the incision was sutured, anesthesia was discontinued, and the mice were transferred to a humidity- and temperature-controlled chamber. Reperfusion was achieved 60 min after occlusion by re-anesthetizing the mice and retracting the filament. Immediately before reperfusion, mice were given no treatment (control; n = 13) or single i.p. injections of vehicle (n = 8), 0.02 (n = 10), 0.2 (n = 8), or 2.0 (n = 8) mg/kg BW245C. The mice were returned to the chamber for 2 h before being transferred to new, clean home cages. During the entire surgical procedure and recovery phase, mice body temperature was maintained at 37.0 ± 0.5 °C. Postoperative analgesia was provided solely by infiltration of the surgical incision at the end of the procedure with 0.1-mg/kg bupivacaine.

Functional outcome tests

On day 4 after the occlusion, mice were tested for different functional outcomes as described below:

1. *Neurologic deficit score:* Neurologic deficit was measured in each mouse on day 4 after reperfusion according to the following 0- to 4-point graded scoring system (Hattori et al., 2000): 0 = no deficit; 1 = forelimb weakness and torso turning to the ipsilateral side when held by tail; 2 = circling to affected side; 3 = unable to bear weight on affected side; and 4 = barrel rolling or no spontaneous activity.
2. *Locomotor activity:* Mice were individually housed in the monitoring cages of the automated Home Cage Video tracking system, which can monitor a maximum of four cages at the same time (Med Associates Inc., St. Albans, VT, USA). After a 15-min habituation, the recording was started and was continued for 15 min. Data were obtained in three sets of 5 min each. Cages were washed and cleaned before and after each testing (Singh et al., 2013).
3. *Cylinder test:* Mice were tested for this task as described earlier (Li et al., 2004) with some modifications. Instead of testing the mice for paw preference, in this study, mice were tested for their spontaneous