Vascular Pharmacology 96–98 (2017) 40–52

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Cerebrovascular recovery after stroke with individual and combined losartan and captopril treatment of SHRsp

John S. Smeda[a,*], Noriko Daneshtalab[b]

[a] Division of BioMedical Sciences, Memorial University of Newfoundland, St. John's, Newfoundland, Canada
[b] School of Pharmacy, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

ARTICLE INFO

Keywords:
Stroke recovery
Middle cerebral artery
Myogenic response
Endothelium
Protein kinase C
Uricaemia
Angiotensin converting enzyme inhibitor
Angiotensin receptor blocker

ABSTRACT

We assessed whether the superior restoration of cerebrovascular function after hemorrhagic stroke by losartan versus captopril treatment was due to better BP, uremia, uricaemia, or aldosterone control in Kyoto Wistar stroke-prone-hypertensive rats and evaluated whether elevated angiotensin II (A2) levels enhanced the effectiveness of losartan treatment. Constriction was studied in the middle cerebral arteries (MCAs) using a pressure myograph. Post-stroke survival increased from 21 to 310 and 189 days respectively with losartan and captopril treatment. Neither treatment reduced BP, both reversed uremia and hyperaldosteronism equally after 7 days. Plasma uric acid remained low. At stroke, MCA constriction to pressure (PDC), protein kinase C (PKC) activation, depolarization, and sarcoplasmic Ca^{2+} were attenuated. Endothelial-dependent-vasodilation by bradykinin and endogenous NO release were lost. Both treatments recovered these functions within 7 days. These functions deteriorated after 116 days of captopril but not losartan treatment. Inhibiting A2 formation during losartan treatment didn't alter BP or vascular recovery. The superior recovery of PDC by losartan over captopril was not produced by better BP, uremia or aldosterone control or elevated A2. PDC recovery was associated with improved PKC function and enhanced basal NO release. The re-establishment of PDC could reduce cerebrovascular over-perfusion and hematoma expansion after stroke.

1. Introduction

Intracerebral hemorrhagic stroke (ICH) is associated with a high incidence of death and survival that does not exceed one week [1]. The development of new cerebral hemorrhages and hematoma expansion are important factors preventing recovery and promoting death after ICH [1,2]. Like humans, Kyoto Wistar stroke-prone spontaneously hypertensive rats (SHRsp) develop ICH spontaneously and survive, on average, only10 days after stroke [3]. Cerebral blood flow (CBF) autoregulation is lost in SHRsp at stroke, and the ability of isolated middle cerebral arteries (MCAs) to appropriately alter tone in response to vascular pressure (a mechanism thought to promote CBF autoregulation) is also abolished [4]. The loss of CBF autoregulation in SHRsp causes brain over-perfusion. This increases vascular shear stress and cerebrovascular pressure which could promote hematoma expansion as well as new hemorrhage formation after stroke [4]. Treatment of SHRsp after stroke with captopril, an angiotensin converting enzyme inhibitor (ACEI) that inhibits angiotensin II (A2) formation, or losartan, an AT-1 receptor blocker (ARB) which blocks the action of A2, restores CBF autoregulation and cerebrovascular pressure dependent constriction (PDC) within a week, and increases the post-stroke lifespan of SHRsp [4]. The treatments differ in that cerebrovascular functions are maintained with losartan treatment, and deteriorate with continuous captopril treatment [4].

There is debate as to the relative benefits of ARB and ACEI use over other drugs in the treatment of hypertension [5]. Human studies have demonstrated that both ARBs and ACEIs are remarkably effective in slowing the progression of kidney disease and represent a preferred treatment of this disease in hypertensive and normotensive patients [5,6]. The cerebrovascular dysfunctions that occur in SHRsp at stroke coincide with kidney disease that produces a reduction in glomerular filtration (GFR) [7,8], uremia [8] as well as the activation of the renin-

Abbreviations: A2, angiotensin II; Ang_{1-7}, angiotensin 1–7; ACE, angiotensin 1- converting enzyme; ACE2, angiotensin - converting enzyme 2; ACEI, angiotensin I - converting enzyme inhibitor; AT-1, angiotensin 2 type-1 (AT-1) receptor; AT-2, angiotensin 2 type-2 (AT-2) receptor; BK_{Ca}, large conductance calcium-activated K^+ channel; BNPDT, basal non-pressure dependent tone; BP, blood pressure; CBF, cerebral blood flow; ICH, intracerebral hemorrhagic stroke; LName, Nω-nitro-L-arginine methyl ester; MCA, middle cerebral artery; NO, nitric oxide; NOS, nitric oxide synthase; PAR2, protease-activated receptor 2; PDC, pressure dependent constriction; PKC, protein kinase C; SR, sarcoplasmic reticulum; SHR, Kyoto Wistar spontaneously hypertensive rat; SHRsp, stroke-prone SHR

* Corresponding author at: Division of BioMedical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St John's, Newfoundland A1B 3V6, Canada.
E-mail address: jsmeda@mun.ca (J.S. Smeda).

http://dx.doi.org/10.1016/j.vph.2017.08.004
Received 1 May 2017; Received in revised form 28 July 2017; Accepted 17 August 2017
Available online 18 August 2017

angiotensin system which elevates plasma A2 and aldosterone [9]. These renal dysfunctions have been implicated in the development of hemorrhagic stroke in SHRsp and humans and may be instrumental in promoting cerebrovascular dysfunction and hematoma expansion. Elevating plasma aldosterone in SHRsp can initiate stroke and enhance death after stroke [9] and the induction of uremia facilitates the loss of cerebrovascular PDC and promotes ICH development in stroke resistant SHR [7]. Reductions in GRF and uremia in humans with kidney disease have been associated with dysfunctions in CBF autoregulation [10], CBF hyper-perfusion [11] and ICH development [12]. In humans, hyperuricaemia increases the risk of stroke development [13] and the superior ability of losartan in reducing the risk of stroke over other antihypertensive therapies has been attributed to a reduction in plasma uric acid [14,15,16]. We believe that the mechanisms by which losartan and captopril restore normal cerebrovascular function and retard death after stroke in SHRsp could be related to the restoration and maintenance normal renal function. One of the objectives of the current study was to evaluated if the more persistent beneficial effects of post-stoke losartan over captopril treatment were due to the superior ability to lower plasma uric acid or control plasma urea and or aldosterone over a 165 day treatment period.

The benefits of losartan and other ARBs over ACEIs have also been attributed to the fact that plasma A2 remains elevated during AT-1 receptor blockade, which enhances AT-2 receptor stimulation [15,16]. AT-2 receptor stimulation during ARB treatment produces multiple positive cerebrovascular effects, which has led to the proposition that ARBs are more effective than ACEIs in preventing stroke in hypertensive humans [15,16]. Therefore another objective of our study was to evaluate whether elevated A2 levels contributed to the beneficial post-stroke effects of losartan over captopril treatment. To test this hypothesis, A2 formation was suppressed during losartan treatment over a 60 day period and the effects of this manipulation on cerebrovascular function were evaluated in relation to SHRsp receiving losartan or captopril monotherapy.

2. Methods

2.1. Stroke development and treatment

Experiments had institutional approval (protocol 14–25-JS) and followed the guidelines of the Canadian Council on Animal Care. Male SHRsp were fed a Japanese style stroke-prone diet containing 4% NaCl (Zeigler Brothers, Gardner, PA, USA) from weaning (5 weeks of age) which caused hemorrhagic stroke to develop between 12 and 18 weeks of age [3]. Stroke was characterized by seizures and severe lethargy as previously outlined [3,4]. SHRsp exhibiting symptoms of stroke were randomly treated with captopril (50 mg/kg/day) or losartan (35 mg/kg/day) delivered in the drinking water. SHRsp from 4 litters were used to determine post-stroke survival and sampled when death was imminent and humane euthanasia was advised. Other rats were sampled prior to stroke (at 10 weeks of age), at stroke onset, and following 7 to 116 days of post-stroke captopril and up to165 days of losartan treatment. The shorter post-stroke lifespan of captopril treated SHRsp (90% mortality at 165 days) limited our ability to extend captopril treatment to 165 days. We did however obtain blood samples from 3 captopril treated rats that survived 165 days of treatment. Separate rats were sampled at stroke and following 60 days of individual and combined post-stroke captopril (50 mg/kg/day) and losartan (35 mg/kg/day) treatment. Combined therapy was expected to produce an inhibition of A2 formation during losartan treatment.

Systolic BP was measured using a tail cuff compression method (IITC, Model 29, pulse/pressure amplifier, Woodland Hills, CA, USA) as previously described [3,4,9]. At sampling, the rats were anesthetized (xylazine (10 mg/kg) + ketamine (50 mg/kg)), blood was taken, and the rats were exsanguinated. The brain was excised, placed in ice cold oxygenated (95% O_2/5%CO_2) Krebs saline and the MCAs were removed for experimentation.

2.2. The measurement of basal tone, pressure dependent constriction, and Total constriction in MCAs

Segments of the left and right MCA at the rhinalis fissure were mounted in a pressure myograph. The changes in lumen diameter (LD) were video recorded at 322× magnification and measured as previously described [17]. The mean pial cerebrovasculature BP of SHRsp is 100 ± 4 mmHg (50% systemic BP) [18]. Therefore, MCAs were equilibrated to 100 mmHg pressure at 37 °C for 30 min. Following equilibration, PDC was inactivated by equilibrating the MCAs slightly above 0 mmHg pressure (to prevent vascular collapse) for 6 min. Subsequently, 100 mmHg pressure was instantaneously reapplied. The reapplication of pressure after the inactivation of PDC caused the MCA lumen to expand. This was followed by constriction to a steady state within 3.5 min. Due to the time frame of the PDC development, significant constriction does not occur until 1 s after the reapplication of pressure. Therefore, the LD at 1 s (following the inactivation of PDC) approximates the LD present at 100 mmHg in the absence of PDC. Constriction from 1 s (prior to the engagement of PDC) to 4 min after the application of a 100 mmHg pressure (completion of constriction to pressure) was used as a measure of PDC in the MCAs. All MCAs maintain a degree of constant basal tone that we have termed basal non-pressure dependent tone (BNPDT) which cannot be abolished by reducing pressure. BNPDT is extensively discussed within the supplement of a previous publication [17]. BNPDT was measured as the % decrease in LD from maximal dilation at 100 mmHg (induced by nifedipine (3 μM)) to the LD present at 1 s after the application of the 100 mmHg pressure step. Total constriction of the MCAs at 100 mmHg (BNPDT + PDC) was calculated as the % decrease in LD from maximal dilation to the LD present 4 min after the application of the 100 mmHg pressure step. Fig. S1 in the online supplement shows a PDC response to a 100 mmHg pressure step in a MCA and provides details describing the measurement of PDC, BNPDT, and total constriction. After measuring PDC, all subsequent experiments were performed at 100 mmHg and (with the exception of high $[K^+]_o$ constriction) at 37 °C.

2.3. Constriction in response to PKC activation, vasopressin and depolarization

MCA constriction to PKC activation by phorbol 12,13-dibutyrate (1 μM) was determined at 100 mmHg in the presence of nifedipine (3 μM). Constriction was totally inhibited by the PKC inhibitors chelerythrine (12 μM) or bisindolylmaleimide (5 μM) [19].

Vasopressin (1.2 μM) constriction was also measured at 100 mmHg in MCAs maximally dilated with nifedipine (3 μM). The sarcoplasmic reticulum (SR) Ca^{2+} store in the MCA smooth muscle is replenished by Ca^{2+} entry through L-type channels. When the latter channel is blocked, vasopressin produces a phasic contractile response corresponding to the release and subsequent depletion of Ca^{2+} from the SR store [20]. This response cannot occur when the SR Ca^{2+} store is depleted with cyclopiazonic acid (10 μM) or Ca^{2+} free 5 mM EGTA Krebs saline and is absent when the stored Ca^{2+} has been previously released by serotonin prior to the application of vasopressin [20].

MCA constriction to depolarization was measured at 100 mmHg pressure by elevating $[K^+]_o$ from 4.6 to 100 mM at 23 °C. At 23 °C, both BNPDT and PDC are inactivated, producing near maximal dilation of the MCAs [21]. This allowed constriction to high $[K^+]_o$ to be measured without the complications of underlying basal and pressure induced tone. Nifedipine (3 μM) reversed constriction indicating that the response was being mediated through L-type Ca^{2+} channels. High $[K^+]_o$ solutions were osmotically balanced by removing Na^+ from the Krebs saline solution.

2.4. The influence of NO on basal tone and dilation to bradykinin

The influence of spontaneous NO release on tone was assessed in