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PERSPECTIVE

Cell-specific mineralocorticoid receptors: future therapeutic targets for stroke?

The mineralocorticoid receptor (MR), well known to be expressed in renal epithelial cells where it is important in fluid and electrolyte homeostasis, has aldosterone as one of its main agonists. Much research in the last 10–15 years indicates that MRs are also expressed outside of the kidney, including in the brain, vasculature and heart, where they contribute to the pathophysiology of disease (Dinh et al., 2012; Jaisser and Farman, 2016). Excess aldosterone is a cardiovascular risk factor, and MR antagonism is beneficial in the setting of cardiovascular disease (both clinically and experimentally), including in experimental stroke, whereby MR antagonism is beneficial in reducing both cerebral infarct size (Iwanami et al., 2007; Oyamada et al., 2008) and cerebral vascular remodeling (reviewed in Dinh et al., 2012) following cerebral ischemia. MR antagonism also reverses remodeling, both during aldosterone/mineralocorticoid excess and following cerebral ischemia. The advent of technology to generate mice which lack specific genes in specific cell types has allowed investigation into the contribution of MR in cell types such as vascular endothelial and myeloid cells to the pathophysiology of cerebrovascular disease and stroke (Frieler et al., 2011, 2012; Dinh et al., 2016). Endothelial cell MRs mediate cerebrovascular oxidative stress and brain inflammation in response to excess aldosterone (Dinh et al., 2016), and myeloid MR contribute to the ischemic damage, inflammation and neurological impairment following cerebral ischemia/reperfusion (Frieler et al., 2011, 2012). These and further investigations into the contribution of cell-specific MRs to cerebrovascular disease and stroke can help guide the future design of therapeutic strategies for stroke treatment.

Background: The MR is a ligand-dependent transcription factor that is a member of the nuclear receptor superfamily, and is bound by the physiological ligands aldosterone and cortisol (in rodents, corticosterone). Due to the higher circulating levels of cortisol and corticosterone compared to aldosterone (~100–1000 times higher), the MR would normally be bound by cortisol. The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11-βHSD2) metabolizes cortisol, thus aldosterone requires the presence of 11-βHSD2 to activate the MR, such as occurs in renal epithelial tissues (where the 'classic' actions of aldosterone, including water and electrolyte homeostasis, are mediated (Dinh et al., 2012; Jaisser and Farman, 2016)), and in vascular endothelial and smooth muscle cells (Dinh et al., 2012).

In addition to renal expression, many studies have reported MR expression in non-epithelial tissues, including in cardiomyocytes, vascular cells (both endothelial and smooth muscle cells), immune cells and brain (Dinh et al., 2016; Jaisser and Farman, 2016). MR expression, which is particularly high in several brain regions (Hawkins et al., 2012) has been reported in endothelial cells, glial cells (including microglia and astrocytes) and neuronal cells (Frieler et al., 2011; Hawkins et al., 2012; Oyamada et al., 2008; Dinh et al., 2016). Ischemic stroke is caused by interruption of blood flow to the brain, thus stimuli which alter both structure and function of cerebral blood vessels can predispose to increased stroke risk. MR activation with deoxycorticosterone acetate reportedly increased the amount of ischemic damage in the brain compared to control treatment, and increased middle cerebral artery wall: lumen ratio and wall thickness, and resulted in smaller lumen and outer diameters compared to control rats, suggesting that MR activation is associated with cerebral vascular remodeling (reviewed in Dinh et al., 2012). Recent reports suggest that excess aldosterone causes oxidative stress, inflammation and endothelial cells dysfunction in the brain (Chrissobolis et al., 2014; Dinh et al., 2016) supporting the concept that excess aldosterone levels are associated with stroke risk (Dinh et al., 2012). Indeed, patients with primary aldosteronism have much higher rates of stroke than sex-, age- and blood pressure-matched essential hypertensives (Dinh et al., 2012). Endothelial cell dysfunction underlies many cardiovascular diseases, including stroke, and may be a key initiating step in these diseases. Cerebral endothelial dysfunction and oxidative stress in the brain and its vasculature has been reported in rodent models of ischemic stroke (reviewed in Chrissobolis et al., 2011). Inflammation, a key component of stroke pathology, is also increased in the brain during aldosterone excess (Dinh et al., 2016).

Beneficial effects of MR antagonism in the cerebral vasculature and during stroke: MR antagonism is beneficial in the cerebral vasculature and brain in models of stroke and aldosterone excess by preventing endothelial dysfunction, oxidative stress and remodeling in the vasculature, as well as brain inflammation. In the stroke-prone spontaneously hypertensive rat (SHRSP), spironolactone treatment improved cerebral vascular endothelium-dependent dilation (McClain and Dorrance, 2014), increased lumen and outer diameter of middle cerebral arteries, and decreased wall/lumen ratio even when hypertension was well established, suggesting that MR antagonism is beneficial in the cerebral vasculature even after pathology has been established (reviewed in Dinh et al., 2012). During aldosterone excess, spironolactone inhibited cerebral vascular oxidative stress and brain inflammation in mice (Dinh et al., 2016).

MR antagonism is beneficial in the setting of ischemic stroke in that the MR antagonists, eplerenone and spironolactone, are both protective in experimental models of cerebral ischemia. Pretreatment with eplerenone not only reduced ischemic area, but it also reduced the neurological deficit in mice subjected to focal middle cerebral artery occlusion (MCAO) (Iwanami et al., 2007). Similarly, spironolactone reduced infarct volume in a mouse model of MCAO (Oyamada et al., 2008). In addition, MR expression was markedly increased in the ischemic striatum following MCAO (Oyamada et al., 2008). MR antagonism, which also reduces oxidative stress in the ischemic areas (Iwanami et al., 2007; Oyamada et al., 2008), thus exerts clear benefits in preclinical models of ischemic stroke.

Cell-specific MR deletion in cerebrovascular disease and ischemic stroke: In keeping with the beneficial effects of MR antagonism in the cerebral circulation and brain during stroke, recent reports of a role for MR expressed in specific cell types has provided additional information on specific targets to offset detrimental outcomes during cerebrovascular disease and stroke. Such research is possible with the recent development of mice lacking MR on specific cell types, such as in endothelial, myeloid and vascular smooth muscle cells.

Endothelial MR-deficient mice: Cerebrovascular oxidative stress in response to aldosterone excess was abolished in mice lacking MR on endothelial cells (Dinh et al., 2016). Aldosterone induced inflammation in the brain, specifically increasing expression of the monocyte chemoattractant proteins chemokine (C-C motif) ligand 7 (CCL7) and CCL8, and this was prevented by endothelial cell-specific MR deletion, suggesting that the endothelial MR may be an important mediator of monocyte/macrophage entry into the brain (Dinh et al., 2016). This provides insight into the potential of targeting endothelial MR for preventing inflammatory cell infiltration into the brain following ischemic stroke, particularly during conditions of aldosterone excess.

Myeloid MR-deficient mice: In male mice lacking MR expressed on myeloid cells, infarct volume following MCAO was markedly attenuated, an effect associated with a reduction of both activated microglia (immunoreactive $Iba1^+$ cells) in the ischemic core, and pro-inflammatory cytokines including interleukin interleukin 1 beta (IL-1β), macrophage inflammatory protein 1 alpha (MIP1α), tumor necrosis factor alpha (TNF)-α and monocyte chemoattractant protein-1/CCL2 in the ischemic hemisphere (Frieler et al., 2011).

A follow-up study by the same group investigated whether myeloid MR deletion protects against cerebral ischemia in females. As in males, female mice were protected from cerebral ischemia (*i.e.*, infarct size was reduced) and elevated pro-inflammatory gene expression, despite eplerenone exerting protection in males only (Frieler et al., 2012). Female mice lacking MR on myeloid cells were protected from neurological deficit following cerebral ischemia (Frieler et al., 2012). These data are consistent with a lack of

effect of both spironolactone and eplerenone on cerebral ischemia in females, albeit in a model of permanent ischemia (reviewed in Dinh et al., 2012). The authors suggest MR antagonists may exhibit sex-specific effects in different cell types (Frieler et al., 2012). This further highlights the importance of investigating MR expression on specific cell types to guide future therapy in stroke prevention, as a currently available MR antagonist such as eplerenone is not protective in females. Future agents that are specific for myeloid MRs may thus be useful in the treatment of stroke in females.

Despite protective effects of MR deletion in myeloid cells of males and females, in myeloid MR-deficient mice subjected to permanent ischemia (intraluminal filament model and a photothrombotic model) there was no reduction in ischemic area, improvement of neurological deficit or reduction in pro-inflammatory markers (Frieler et al., 2012), thus suggesting the protective effects of MR deletion are selective to reperfusion injury. Perhaps pathology during permanent occlusion is largely mediated by resident immune cells within the brain without much influence of infiltrating peripheral immune cells (marked brain inflammation was reported following permanent occlusion (Frieler et al., 2012)). During transient ischemia, infiltrating immune cells expressing MR from the periphery largely mediate ischemic damage.

Vascular smooth muscle cell MR-deficient mice: To our knowledge, the effect of MR deficiency in vascular smooth muscle of the cerebral vasculature and during ischemic stroke has not been investigated. The importance of such investigations is highlighted by the recent finding that vascular smooth muscle cell MR modulates blood pressure during aging and in response to angiotensin II, as well as vascular contractile function (McCurley et al., 2012). Exacerbated hypertension during aging due to excessive vascular smooth muscle MR function highlights the potential targeting of MR expressed in this cell type to decrease blood pressure and therefore stroke risk.

MR antagonists: The steroidal MR antagonists spironolactone and eplerenone are currently approved for the treatment of hypertension and heart failure (Jaisser and Farman, 2016); however, both have limitations. For example, spironolactone (the first MR antagonist developed) produces side effects such as impotence, gynecomastia and menstrual irregularities owing to its poor selectivity profile in that it also inhibits androgen and progesterone receptors. Eplerenone, a second-generation MR antagonist, is more selective for MRs than spironolactone, but is less potent than spironolactone with 40-fold lower affinity for MR. MR antagonists are also associated with hyperkaliemia, which can limit their use (Jaisser and Farman, 2016). Recently, BAY 94-8862 (a non-steroidal compound known as finerenone), a third-generation MR antagonist has been developed and is currently undergoing clinical trials (for heart failure and diabetic nephropathy), with promising initial results (reviewed in Jaisser and Farman, 2016).

Future directions: The above evidence demonstrates the beneficial effects of broad spectrum MR antagonism on cerebral vascular dysfunction, oxidative stress and inflammation, which extend to their beneficial effects in the setting of cerebral ischemia, both in terms of reducing the impact of ischemia on associated brain infarct development and oxidative stress, and neurological dysfunction. What is also clear is that there are still unanswered questions with much work to be done in the pursuit of more specific targets to guide drug therapy in stroke. For example:

• What is the role of endothelial and vascular smooth muscle MR in mediating ischemic injury, brain inflammation and neurological deficits in MCAO, and how are these impacted by sex differences and permanent *vs.* transient ischemia?

• What are the roles of endothelial, vascular smooth muscle and myeloid MR in mediating the deleterious actions of aldosterone excess in the cerebral vasculature and post-stroke, and are there any sex differences? The impact of sex differences on aldosterone-induced cerebral vascular abnormalities and aldosterone-induced cerebral ischemia is, to our knowledge, unknown.

• What is the impact of the more recently developed MR antagonist BAY 94-8862 in the SHRSP and mouse models of cerebral ischemia, and is it protective in females?

The development of therapeutic agents to target MR on specific cell types may represent a promising way forward in stroke therapy.

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Quynh N. Dinh, Grant R. Drummond, Christopher G. Sobey, Sophocles Chrissobolis*

Vascular Biology & Immunopharmacology Group, Cardiovascular Disease Program and Department of Pharmacology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia (Dinh QN, Drummond GR, Sobey CG)
Department of Pharmaceutical and Biomedical Sciences, Raabe College of Pharmacy, Ohio Northern University, Ada, OH, USA (Chrissobolis S)

***Correspondence to:** Sophocles Chrissobolis, Ph.D., s-chrissobolis@onu.edu.*
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