L-lactate reduces ischemia-induced oligodendrocyte injury and modulates white matter HCA1 expression in a mouse MCAO model

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INTRODUCTION

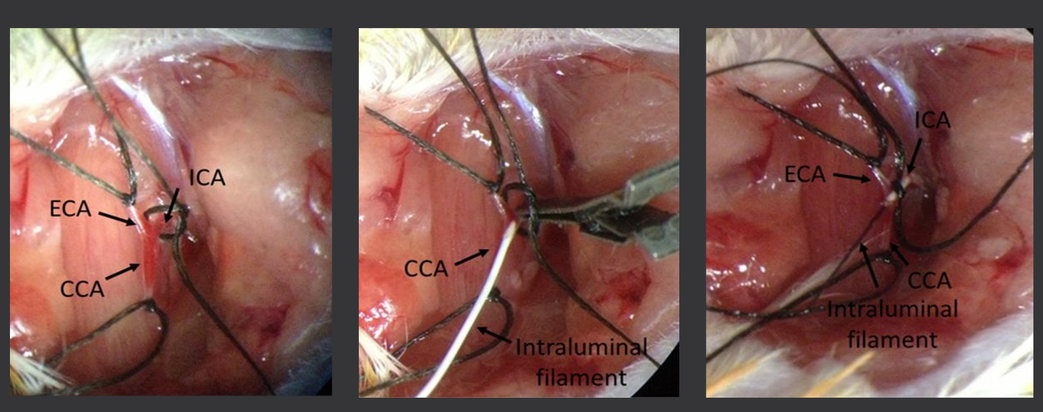
Stroke is a leading cause of death and long-term disability. In ischemic stroke, arterial occlusion interrupts cerebral blood flow and substrate delivery to brain tissue, resulting in multicellular death due to energy failure. An ischemic core of necrotic cell death immediate to the occluded artery is surrounded by a peri-infarct penumbra where energy metabolism is preserved due to a less severe blood flow reduction. While cells within the ischemic core are irreversibly injured, tissue within the penumbra may be salvaged by timely recanalization, making the penumbra the target for several neuroprotective therapies.

Lactate is a multifunctional metabolite that is involved in several physiological and pathophysiological processes in the brain. Lactate can serve as a supplemental fuel for neuronal utilization during increased metabolic demand and has been shown to recover synaptic function, reduce neuronal death and improve functional outcomes in models of hypoglycaemia, hypoxia and ischemia. Besides its metabolic role, lactate has recently emerged as an important signalling molecule, regulating several neuronal functions via the lactate receptor hydroxycarboxylic acid receptor 1 (HCA1). Modulation of HCA1 by lactate has been shown to be associated with neuronal protection in a model of cerebral ischemia.

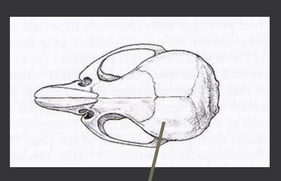
Knowing the urgency of providing both neuronal and glial protection during ischemia, we assessed whether lactate protection extends beyond gray matter in a commonly used model of focal ischemia, the middle cerebral artery occlusion (MCAO) model.

METHODS

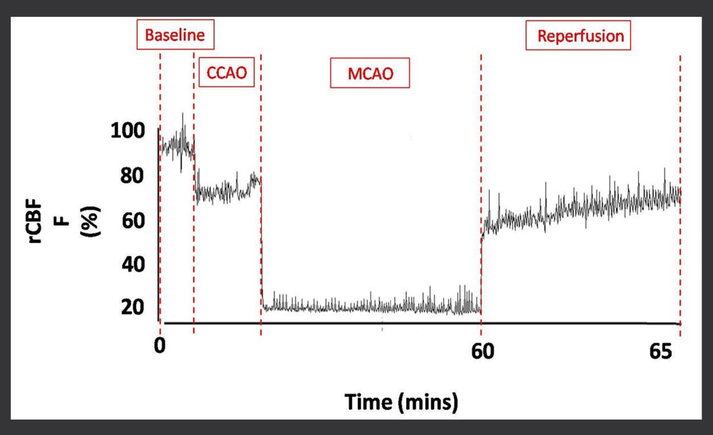
1. Ischemia-reperfusion was induced using the mouse intraluminal filament MCAO model, which closely mimics human ischemic stroke and results in neuropathological, gliopathological and behavioural consequences.
2. Following anaesthesia induction, occlusion of the left MCA was induced in CD-1 mice. First, the left common carotid artery (CCA) and external carotid artery (ECA) were isolated and permanently ligated. An arteriotomy was performed on the CCA and a silicon-coated monofilament was introduced into the CCA and advanced orthogradely through the ICA to occlude the origin of the MCA.



3. A 60min occlusion period was allowed, after which the filament was withdrawn to allow recanalization.
4. Correct suture placement and successful occlusion were confirmed via Laser Doppler Flowmetry monitoring of relative cerebral blood flow over the left MCA territory.



LDF probe placement over the left MCA territory ~2mm caudal, 5mm lateral to bregma



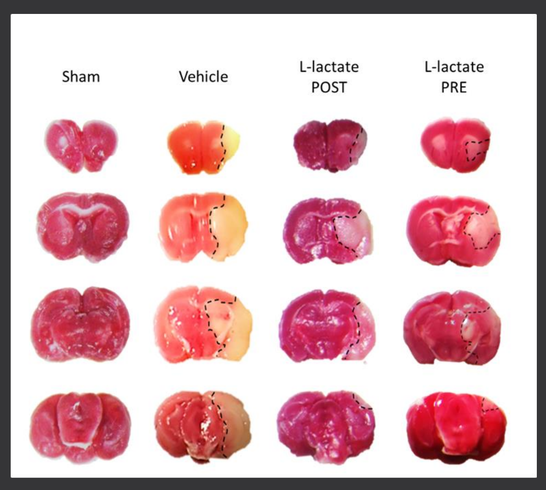
5. L-lactate (250mgkg^{-1} BW) or vehicle were injected intraperitoneally 5-10 minutes prior to occlusion or upon reperfusion.
6. Functional outcomes were assessed using the Modified Bederson Score (MBS) as a score to assess global neurological deficit, the Corner test as a measure of forelimb asymmetry and the Wire Hanging test to assess grip strength and endurance.
7. Ischemic lesion volumes were assessed histologically by 2, 3, 5,-triphenyltetrazolium chloride (TTC) staining, a mitochondrial stain which stains viable tissue red while infarcted tissue remains unstained (white).
8. Immunohistochemical analyses were performed using antibodies against CNPase (mature oligodendrocytes, OLs), HCA1 (HCA1 receptor), glial fibrillary acidic protein (GFAP, astrocytes) and Hoechst 33342 as a nuclear counterstain

CONCLUSION

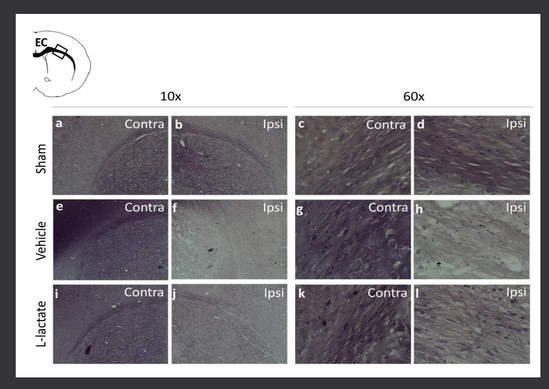
Besides providing neuroprotection, L-lactate could mitigate ischemic injury in WM components in this commonly used mouse model of MCAO. The identification of the lactate receptor, HCA1 on WM oligodendrocytes and astrocytes and receptor modulation during ischemia and by exogenous lactate presents a novel role for lactate signalling and glial crosstalk in WM which could potentially be involved in pathophysiological or protective pathways.

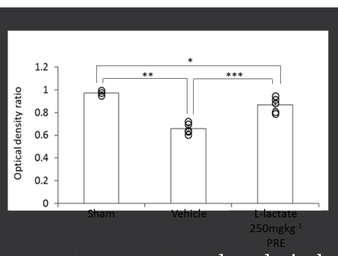
RESULTS

HISTOLOGY



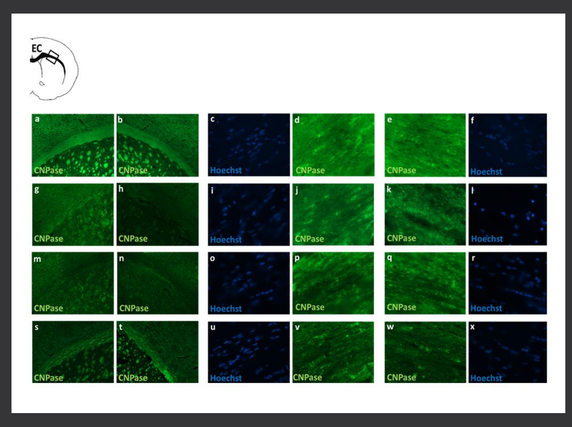
- Lactate pre- or post-treatment resulted in reduced lesion size, edema, brain water content in CD-1 mice
- Comparable rCBF, physiological parameters vs vehicle

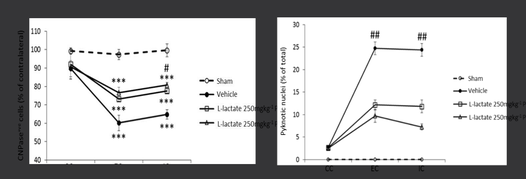




- Lactate pre-treatment reduced ischemia-induced structural aberrations and myelin pallor observed as more intense LFB staining and compared to vehicle
- Optical density ratios were higher in the lactate group, suggestive of improved myelin condition with lactate treatment

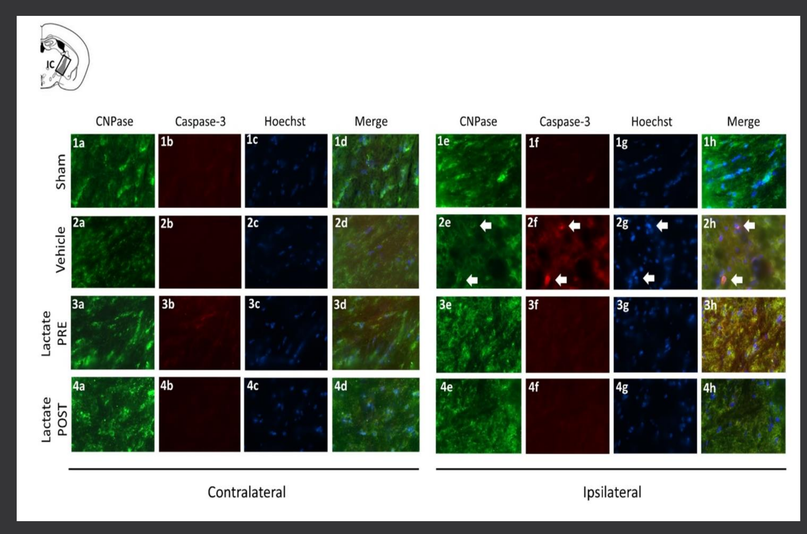
IMMUNOHISTOCHEMISTRY

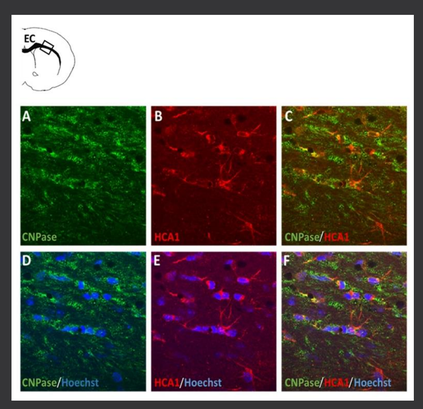




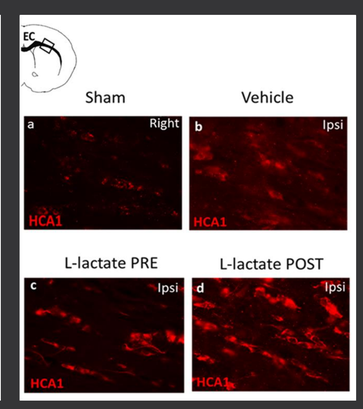
- Pre- or post-treatment with L-lactate resulted in a decreased loss of CNPase$^+$ cells and a reduced appearance of pyknotic nuclei in ipsilateral WM regions compared to vehicle treatment at acute and subacute stages of ischemia

- Lactate delivered before or after ischemia resulted in reduced activation of the pro-apoptotic protein caspase-3 on WM OLs, particularly in the internal capsule, compared to vehicle

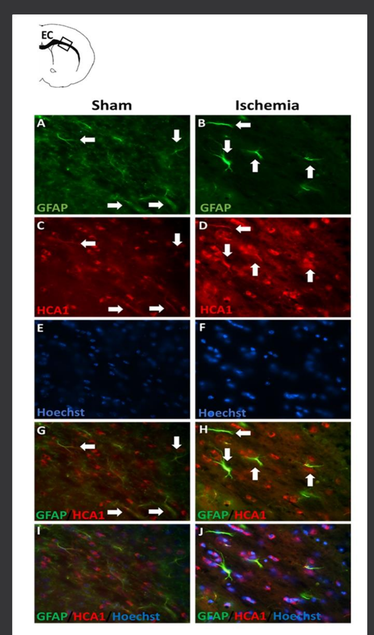




- The lactate receptor hydroxycarboxylic acid 1 (HCA1), which, to date, has been reported to be expressed in several gray matter regions of the brain and is thought to be involved in lactate-mediated neuroprotection, was found to also be expressed on somata of WM OLs as shown by double-labelling immunohistochemistry of CNPase$^+$ OLs (green) and HCA1 (red). Merged images additionally show Hoechst-stained nuclei (blue)



- HCA1 fluorescence expression on ipsilateral WM OLs was observed to increase during ischemia and was further enhanced by exogenous L-lactate



- HCA1 fluorescence expression was also observed on resting astrocytes and astrocytes activated during ischemia in WM regions