Preconditioning with interleukin-1 alpha is required for the neuroprotective properties of mesenchymal stem cells after ischaemic stroke in mice

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Abstract

Mesenchymal stem cells (MSCs) pre-conditioning with interleukin-1 alpha (IL-1α) drives MSCs toward a potent anti-inflammatory and pro-trophic phenotype. The aim of this study was to assess the therapeutic potential of IL-1α preconditioning of MSCs, administered intra-arterially (a clinically relevant approach in the setting of thrombectomy) after experimental cerebral ischaemia in mice.

Focal ischaemic stroke was induced by filament occlusion of the middle cerebral artery in mice. After 3 h from start of occlusion, animals were treated with vehicle, $9.1 \times 10^4$ non-conditioned or IL-1α preconditioned MSCs by intra-arterial administration. Animals were allowed to recover for 3 days or 14 days post-stroke and lesion volume and functional outcomes were evaluated. To assess reperfusion cerebral blood flow was measured at 1.5 h after treatment using laser speckle imaging in a separate cohort of animals.

Preconditioned MSCs reduced lesion volume and neurological deficits compared to vehicle by 67%, while non-conditioned MSCs had no effect, at 3 days post-stroke. A separate cohort of animals recovered to 14 days post-stroke also showed reduced infarct volume at 48 h (assessed by MRI) when treated with preconditioned MSCs, along with lower neurological deficits at 14 days and better weight recovery compared to vehicle treated mice. Cerebral blood flow was increased by preconditioned MSCs compared to vehicle by 32%.

Preconditioning MSCs with IL-1α increases their neuroprotective capability and improves functional recovery after delayed intra-arterial administration in a mouse model of focal cerebral ischaemia. With increasing use of thrombectomy the adjunct use of preconditioned MSCs therefore represents a highly relevant therapy to improve outcomes in ischaemic stroke.

Introduction

With increasing use of effective intra-arterial thrombectomy treatment, both the Stroke Treatment Academic Round Industry Roundtable (STAIR) X consortium\(^1\) and the 4th Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS)\(^2\) meetings recommend greater investigation into the integration of endovascular thrombectomy with other therapeutics, such as cell therapy. Meta-analysis shows mesenchymal stem cells (MSCs) to be beneficial in terms of reducing infarct damage and improving functional outcomes in experimental stroke models\(^3\), and there are several ongoing or completed early phase feasibility, dose ranging or phase 2 clinical trials in stroke\(^4\). However, a recent study in comorbid animals showed that non-conditioned MSCs failed to exert neuroprotective actions after experimental stroke\(^5\). Our previous work has shown MSCs preconditioned with interleukin-1 alpha (IL-1α) adopt a potent anti-inflammatory and pro-trophic phenotype, which may translate into a more effective therapy for stroke\(^6\). Here we show for the first time that IL-1α preconditioning is required to reveal neuroprotective properties of MSCs and that intra-arterial (IA) administration of IL-1α preconditioned MSCs, alongside thrombectomy, could potentially be a highly effective therapeutic approach for ischaemic stroke.
Methods

Experimental Design

Animal procedures were carried out in accordance with the Animal Scientific Procedures Act (1986) and the European Council Directive 2010/63/EU and were approved by the Animal Welfare and Ethical Review Body of the University of Manchester, UK. Experiments followed ARRIVE\textsuperscript{7} and IMPROVE guidelines\textsuperscript{8}. See methods section in supplementary information for details of blinding, randomisation and exclusions.

Animals

Mice were male C57BL/6 (Charles River, UK), aged 12 to 14 weeks at the time of surgery. A total of 36 animals (12 mice per experimental group including vehicle-treated, IL-1α conditioned MSCs-treated and non-conditioned MSCs-treated groups) underwent surgery in the short term experiment (3 day end-point) and 20 animals (10 mice per group; vehicle-treated and IL-1α conditioned MSCs-treated) for the longer term (14 day) experiment. In a biodistribution and cerebral blood flow experiment, 33 animals (11 mice per experimental group including vehicle-treated, IL-1α preconditioned MSCs-treated and non-conditioned MSCs-treated groups) were allocated to receive labelled MSCs for histological biodistribution analysis and underwent laser speckle imaging at 1.5 h after treatment, prior to culling 30 mins later to retrieve brains for MSC biodistribution analysis (2 h after treatment).

Mesenchymal stem cell culture

Human bone marrow derived MSCs (fetal source 20–22 weeks old) were purchased from 3H Biomedical (Sweden) and used at culture passages 4–6. MSCs were cultured and preconditioned with recombinant human IL-1α (10ng/ml, R&D Systems, UK) in serum free MesenPro for 5 min as previously described\textsuperscript{6}. MSCs designated for biodistribution experiments had an additional stage of labelling with CellTracker™ Deep Red dye. After preconditioning with IL-1α or vehicle (serum-free MesenPro) and detached by trypsinisation, MSCs were labelled by incubation with CellTracker™ Deep Red dye for 15 mins in suspension (5µM, serum-free MesenPro) before removing excess dye and washing. IL-1α conditioned MSCs (as well as non-conditioned MSCs) for administration in mice were prepared on the day of surgery, kept on ice for up to 3 h and then warmed to room temperature before administration.

Focal cerebral ischaemia and MSC treatment

Transient focal cerebral ischaemia was induced by middle cerebral artery occlusion (MCAO) for 30 mins, based on our previously published protocol\textsuperscript{9}. At 3 h from start of occlusion, animals were treated under isoflurane anaesthesia with vehicle (serum-free Mesenpro media), 9.1x10\textsuperscript{4} IL-1α conditioned MSCs or 9.1x10\textsuperscript{4} non-conditioned MSCs by IA infusion (20 µl cell suspension, 0.5 µl/sec), via the filament incision site. Animals were recovered and returned to normal housing prior to behavioural testing.

Laser Speckle imaging
Animals receiving labelled MSCs underwent laser speckle imaging at 1.5 h post-treatment (4.5 h post-stroke) to measure cerebral blood flow (CBF). Mice were anaesthetised with isoflurane (4% in 30% O\textsubscript{2} and 70% N\textsubscript{2}O) and fixed in a stereotactic frame with ear bars and mouth bar to prevent movement. The scalp was exposed by a mid-line skin cut and the blood flow recorded for 5 mins.

**Magnetic Resonance Imaging**

In the longer-term experiment (14 days post-stroke), mice underwent magnetic resonance imaging (MRI) scans at 48 h post-stroke. Animals were anaesthetised with 4% isoflurane and T2-weighted scans were conducted on Bruker Advance III console (Bruker Biospin Ltd, UK) using a 7 Tesla magnet. A total of 14 serial slices with a thickness of 1 mm were acquired. Lesion volumes were measured using ImageJ and corrected for oedema.

**Tissue processing, histology and Immunofluorescence**

See methods section in supplementary information for details on tissue processing.

**Functional outcomes and statistical tests**

See methods section in supplementary information for details of behavioural tests and statistical analyses.

**Results**

All animals survived to the end time point of 3 days post-stroke in the short-term experiment and were included in analysis. After delayed IA infusion, preconditioned MSCs reduced lesion volume (67%, \(p = 0.002\), Fig. 1A) and neurological deficits (52%, \(p = 0.002\), Fig. 1B) compared to vehicle at 3 days post-stroke. In the biodistribution and CBF experiment, preconditioned MSCs increased CBF in the ipsilateral hemisphere when compared to vehicle (32%, \(p = 0.0344\), Fig. 1C) but not unconditioned MSCs. There was no difference in the number of MSCs found in the ipsilateral side of the brain between unconditioned and preconditioned MSC treatment (Fig. 1D).

To investigate longer-term effects of IL-1\(\alpha\) preconditioning on MSCs, animals were recovered to 14 days post-stroke. MRI at 48 h confirmed our finding from the short-term experiment that preconditioned MSCs were neuroprotective, reducing lesion volume by 51% compared to vehicle at 48 h post-stroke (Fig. 2A, \(p = 0.003\)). In addition, animals treated with preconditioned MSCs showed improved neurological score (Fig. 2B, \(p = 0.001\)) at 14 days and more rapid recovery of body weight (Fig. 2C, \(p = 0.004\)) compared to vehicle treated mice. Secondary outcomes of general wellbeing (burrowing, nest building, Fig. 2D-2E) and the cylinder test showed no differences between treatment groups (Fig. 2F).

**Discussion**

Thrombectomy has opened a great opportunity for adjunct treatments, which cell therapy is uniquely suited to, by allowing a more precise route to the area of infarct with a more optimal dose. IA
administration allows a greater number of cells to reach the ischaemic penumbra, bypassing filtering organs, such as the lung, liver and spleen, allowing cells to traverse into the intraparenchymal space from the intravascularure\textsuperscript{10}.

Previous studies have explored \textit{in vitro} approaches to enhance the therapeutic potential of MSCs, such as pharmacological preconditioning, molecular priming, hypoxic preconditioning, tissue engineering and growth medium\textsuperscript{11}. This study demonstrates for the first time that preconditioning of MSCs with IL-1\(\alpha\) is required to induce their neuroprotective properties in a MCAO model of stroke. Importantly, to confirm clinical translatability in the context of intra-arterial thrombectomy, the preconditioned MSCs were administered 3 h from start of occlusion and given intra-arterially, which is more efficient in targeting delivery of cells to the site of injury compared to intravenous (IV) administration and requires lower doses\textsuperscript{12}. Stem cell delivery by IA administration is already well characterised clinically in terms of safety and efficacy\textsuperscript{13}, with several ongoing trials\textsuperscript{4}.

The therapeutic effects of MSCs have been mainly attributed to paracrine actions of their secretome; consisting of multiple cytokines, morphogens, small molecules and exosomes\textsuperscript{11}. We have previously shown that the MSC secretome can be polarised toward a more anti-inflammatory and pro-trophic phenotype by IL-1\(\alpha\)\textsuperscript{6}. This study shows that early neuroprotection conveyed by preconditioned MSCs could be in part attributed to increasing blood flow to the infarct area. Furthermore, the long-term study indicates that beneficial actions of preconditioned MSCs occur primarily in the acute phase post stroke. A possible mechanism of MSCs altering CBF may be due to their ability to secrete vascular endothelial growth factor (VEGF)\textsuperscript{14}, which is a potent vasodilator\textsuperscript{15}. This study demonstrates however that the neuroprotective mechanisms triggered by IL-1\(\alpha\) preconditioning of MSCs is likely to be multi-factorial.

In summary, our results demonstrate preconditioning MSCs with IL-1\(\alpha\) increases their therapeutic neuroprotective properties after delayed IA administration in transient focal cerebral ischaemia and could be an effective adjunctive strategy alongside intra-arterial thrombectomy.

**Declarations**

**Acknowledgements**

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**Conflicts of Interests**
None to disclose

References


Figures

Figure 1

Effect of IL-1 pre-conditioned and non-pre-conditioned MSCs on short-term outcomes. A short-term experiment with an end point of 3 days post-stroke showed a reduction in (A) Lesion volume, ** p= 0.002 (Cresyl Violet staining) and (B) Neurological deficits (neurological deficit scoring), ** p= 0.002 when
compared to vehicle. In a separate experiment, MSCs were labelled with CellTracker™ Deep Red dye for biodistribution analysis and laser speckle imaging was used to measure the effects of MSC preconditioning on cerebral blood flow (CBF). (C) Preconditioned MSCs increased CBF in the infarct region (represented as % blood flow to the contralateral region, measured at 1.5 h after dosing) when compared to vehicle * p= 0.0344, while non-preconditioned MSCs did not. At 30 mins after laser speckle imaging (2 h after dosing), brains were harvested and labelled MSCs were counted after immunofluorescence imaging. (D) MSCs were found in cerebral vasculature of the ipsilateral side of the brain (arrows indicate MSCs in vessels, scale bar is 25 µm), but IL-1α preconditioning did not alter biodistribution. Vehicle was MesenPro media, MSCs were injected intra-arterially at 9.1x10⁴ 3 h after the start or occlusion. Data expressed as mean ± SEM (A, C and D) or median ± IQR (B).
Figure 2

Long term effect of IL-1α preconditioned MSCs on stroke outcome measures at 14 days post-stroke. IL-1α preconditioning on MSCs reduced (A) Lesion volume (at 48 h using MRI imaging), ** p= 0.003 (B) Neurological deficits (neurological deficit scoring), p= 0.001 (C) Body weight loss, *** p= 0.004, while (D) Burrowing (E) Nesting (F) Cylinder test remained neutral between treatment groups. Vehicle was
MesenPro media, MSCs were injected intra-arterially at 9.1x10^4 3 h after the start of occlusion. Data expressed as mean ± SEM (A, C, D and F) or median ± IQR (B+E).

**Supplementary Files**

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- Wongetal10.11.22versionSupplementary.docx