The Relationship Between Cerebral Reperfusion And Regional Expression Of Matrix Metalloproteinase-9 In Rat Brain Following Focal Cerebral Ischemia

A. S. Douglas, a,b* J. A. Shearer, a,b A. Okolo, a,b A. Pandit, b M. Gilvarry c and K. M. Doyle a,b

a Department of Physiology and Galway Neuroscience Centre, School of Medicine, National University of Ireland, Galway, Ireland
b CÚRAM–Centre for Research in Medical Devices, National University of Ireland Galway, Galway, Ireland
c Cerenovus, Galway, Ireland

Abstract—We investigated the effect of full and partial mechanical reperfusion on MMP-9 expression in rat brain following middle cerebral artery occlusion, mimicking mechanical thrombectomy. Using percentage hemispheric lesion volume and oedema as measures, partial reperfusion reduced extent of brain damage caused by MCA occlusion, but the protective effect was less pronounced than with complete reperfusion. Using ELISA quantification in fresh frozen tissue, confirmed by immunofluorescence in perfusion fixed tissue, increased MMP-9 expression was observed in infarcted tissue. MMP-9 was increased in lesioned tissue of the anterior and posterior temporal cortex and underlying striatal tissue, but also the normal appearing frontal cortex. No significant increase in MMP-9 in the hippocampus was observed, nor in the unlesioned contralateral hemisphere. Both partial reperfusion and full reperfusion reduced the regional MMP expression significantly. The highest levels of MMP-9 were observed in lesioned brain regions in the non-reperfused group. MMP-9 expression was evident in microvessels and in neuronal cell bodies of affected tissue. This study shows that MMP-9 brain levels are reduced relative to the extent of reperfusion. These observations suggest targeting early increases in MMP-9 expression as a possible neuroprotective therapeutic strategy and highlight the rat MCA occlusion model as an ideal model in which to study candidate therapeutics. © 2020 The Author(s). Published by Elsevier Ltd on behalf of IBRO. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Key words: MMP-9, ischaemic stroke, MCA occlusion rat model, reperfusion, hemispheric lesion volume.

INTRODUCTION

Stroke is a serious and debilitating condition resulting in high mortality and long-term disability in those affected. Stroke represents a significant global burden affecting approximately 17 million people annually and resulting in over 5 million deaths, accounting for 10% of all deaths worldwide (Feigin et al., 2014). Ischaemic strokes, which account for approximately 85% of all strokes, are caused by a blood clot in a cerebral artery resulting in an occlusion and subsequent distal reduction in cerebral blood flow.

Current therapeutic approaches for ischaemic stroke include mechanical thrombectomy and/or the use of a thrombolytic drug, recombinant tissue plasminogen activator (rtPA). rtPA can dissolve blood clots (Disorders and Group, 1995) and has been in clinical use since being approved by the US Food and Drug Administration in 1996 (Zivin, 2009). More recently, following seminal clinical trials in 2014/2015, mechanical thrombectomy, in which the clot is retrieved by endovascular intervention, has become a mainstream clinical intervention (Wahlgren et al., 2016). Timely reperfusion can minimise injury and improve functional recovery. However, reperfusion may also lead to abnormal permeability of the blood brain barrier (BBB) and increased risk of haemorrhagic transformation, due to extravasation of blood components from structurally compromised capillaries into infarcted brain tissue, causing clinical deterioration and death in some patients (Khatri et al., 2012).

Matrix metalloproteinases (MMPs) constitute a family of calcium (Ca\(^{2+}\)) and Zinc (Zn\(^{2+}\)) dependent proteolytic enzymes which are involved in the breakdown of the extracellular matrix (Bode and Maskos, 2003; Djuric and Zivkovic, 2017). MMPs such as...
as MMP-9 (gelatinase B) degrade proteins of the extracellular matrix that are important components of the capillary basal lamina, such as type IV collagen, and lammin (Morodomi et al., 1992). MMP-9 is an inducible enzyme with a bimodal pattern of elevation following a stroke (Yang and Rosenberg, 2015). Initially, MMP-9 can contribute to ischemic injury during a stroke, but it is also necessary for revascularisation and recovery in the days and weeks following the ischemic event (Yang and Rosenberg, 2015). Early increased expression of MMP-9 has been implicated in many adverse events including BBB dysfunction (Gasche et al., 2001; Turner and Sharp, 2016), oedema and HT (Zhao et al., 2006), apoptosis (Copin et al., 2005) and neuronal damage (Lee et al., 2004).

Elevated MMP-9 expression has been reported in human brain tissue following ischaemic and haemorrhagic stroke, primarily localised around blood vessels in the infarct core and associated with activation of microglia in the infarct core and peri-infarct region (Rosell et al., 2006).

It was of interest to mimic mechanical thrombectomy in the rat middle cerebral artery (MCA) occlusion model of stroke to assess the effect of partial or full reperfusion on MMP-9 expression in brain tissue. We examined MMP-9 expression in multiple brain regions following 2 h of MCA occlusion, followed by a further 2 h of full, partial, or no reperfusion by removing the blockage to cerebral blood flow.

EXPERIMENTAL PROCEDURES

Ethical statement

All animal experiments were carried out in accordance with the guidelines of the Animal Care and Research Ethics Committee, National University of Ireland Galway, under licence from the Irish Health Products Regulatory Authority and in strict compliance with the European Communities Council directive 2010/63/EU.

Animals

Male adult Sprague Dawley rats (n = 45 in total; weight 380–410 g) were obtained from Charles River, UK. Rats were group housed (2–3 per cage) in transparent plastic bottomed cages (45 × 25 × 20 cm) containing wood shavings as bedding at a constant temperature (20 ± 2 °C) on a 12–hour light/12–hour dark cycle with ad libitum access to water and food. Rats were allowed to habituate for seven days prior to surgery.

Design of partial occluder

For this study we used a novel occluder designed and manufactured by our industrial partners Neuravi Ltd. This occluder was composed of 2 parts: an outer length of PTFE tubing (OD 0.36 mm; ID 0.20 mm) and an inner nitinol wire (0.10 mm thickness) which was coated with silicone at its terminus (see Fig. 1).

Middle cerebral artery occlusion model

In all experiments, terminal anaesthesia was induced and maintained throughout. Male Sprague Dawley rats were anaesthetised by inhalation of isoflurane (induced at 5% in 100% oxygen at 1 L min⁻¹). Animals were artificially ventilated (1.2 ml per 100 g body weight; 50–60 breaths per minute) with 1.5–2.5% isoflurane in 20% O₂:80% medical air at 0.5 L min⁻¹ to maintain normal physiological parameters. Suitable depth of anaesthesia was assessed by lack of a pedal withdrawal reflex or corneal reflex and this depth of anaesthesia was maintained throughout the procedure. Body temperature was monitored continuously by a rectal probe and maintained at 37 ± 1.0 °C using a homeothermic blanket. The femoral artery was cannulated with polyethylene tubing (OD 0.8 mm; ID 0.4 mm) containing heparinised saline (50 U⁻¹ mL⁻¹) to allow continuous blood pressure monitoring and blood withdrawal for measurement of blood glucose and arterial blood gases. Blood glucose (Accu-chek Aviva monitor) was measured in each animal following arterial cannulation. Arterial blood gases (i-STAT-1 analyser, Abbott Laboratories) were measured repeatedly throughout the procedure, prior to occlusion, and during the occlusion and reperfusion period. Cerebral Blood Flow (CBF) was monitored continuously using a laser Doppler probe (Moor Instruments, Axminster, UK) cemented to the exposed skull above the cortical region perfused by the MCA, at approximately 3 mm posterior to bregma and 6 mm lateral to the midline. Cerebral blood flow was recorded at 10 min intervals and mean values for the occlusion and reperfusion periods were expressed as a percentage of the baseline value.

Permanent middle cerebral artery occlusion (MCAO) was induced by insertion of an occluder (Fig. 1), via an arteriotomy in the left external carotid artery, which was advanced approximately 2 cm into the internal carotid artery to occlude the origin of the middle cerebral artery (Longa et al., 1989) and secured in place by a microvascular clip placed on the internal carotid artery. After 2 h of occlusion, animals underwent either full, partial, or no reperfusion for a further 2 h period. Full reperfusion was produced by withdrawal of the full occluder via the external carotid artery and restoration of perfusion through the common carotid artery. For partial...
reperfusion only the internal component of the occluder was removed while the external tubing remained in position in the blood vessel. Where reperfusion was not performed the occluder was left in place until the end of the experiment. Sham operated animals underwent the MCAO surgery with occlusion of the common carotid artery for 2 h, without insertion of the occluder, followed by a further 2 h period (n = 6).

Tissue collection

At the end of the experiment, a subset of the animals (n = 30) were killed by exsanguination and decapitation and fresh frozen tissue collected for analysis. Sham (n = 6), full reperfusion (n = 12), partial (n = 6) or no reperfusion (n = 6) The brains were carefully removed for analysis of infarct volume and MMP-9 expression by ELISA.

In a separate subset (n = 15) rats were killed by overdose with sodium pentobarbital (ip) and tissues were fixed by cardiac perfusion with saline followed by 4% paraformaldehyde and processed for immunofluorescent analysis of MMP-9 (n = 4–6 per group).

Infarct and oedema quantification in 2, 3, 5-Triphenyltetrazolium chloride (TTC) stained tissue

Following tissue collection, brains were stored on ice and cut into 2 mm thick coronal sections in a brain matrix. Sections were incubated in a 1% (w/v) solution of 2, 3, 5-Triphenyltetrazolium chloride (TTC) in Phosphate Buffered Saline (PBS) at 37 °C for 20 min in the dark to visualise the region of infarct. Stained sections were washed in cold PBS, placed into a six well plate and imaged using a Leica M651 ophthalmic surgical microscope (×10 magnification).

Immediately after imaging, sections were grossly dissected to collect tissue from the following regions; frontal cortex, striatum, anterior temporal cortex, hippocampus, posterior temporal cortex, and the remaining tissue from other regions. Dissection was performed in both the left (ipsilateral) and right (contralateral) hemispheres. Tissue was frozen and stored at −80 °C to await further processing.

Calculation of hemispheric lesion and brain oedema volumes following MCAO and reperfusion

Hemispheric lesion and brain oedema volumes were calculated as described by Li et al. (2004). Briefly, using Image analysis software (Image J), outlines of the hemispheres were manually traced on individual slices, having used neuroanatomical markings to delineate the midline. Area of the infarcted regions (white tissue, Fig. 3 (a)) and of ipsilateral and contralateral hemispheric areas were calculated for individual coronal slices, then summed and multiplied by slice thickness to get the total infarct volume. Percentage hemispheric lesion volume (%HLV) was calculated by the equation:

\[ \% \text{HLV} = \left[ \frac{L_V - (I_V - C_V)}{C_V} \right] \times 100 \]

where \( L_V \) = Ischemic lesion volume; \( I_V \) = volume of ipsilateral hemisphere; \( C_V \) = volume of contralateral hemisphere.

The percentage brain oedema volume was calculated using the formula:

\[ \% \text{Oedema} = \left[ \frac{(I_V - C_V)}{C_V} \right] \times 100 \]

where \( I_V \) = volume of ipsilateral hemisphere; \( C_V \) = volume of contralateral hemisphere.

PROTEIN ANALYSIS IN FROZEN BRAIN TISSUE

Tissue homogenisation

Frozen brain tissue from each region was homogenised in NP-40 lysis buffer (137 mM Sodium Chloride, 20 mM Trizma HCl, 10% v/v Glycerol, 1% v/v Igepal (NP-40)) to give a final protein concentration of 20 mg/ml. Samples were homogenised using a hand held homogeniser on ice for 5 min, centrifuged for 20 min at 14000g at 4 °C and the supernatant was collected and stored at −80 °C to await protein analysis.

MMP-9 ELISA

Total MMP-9 expression in each brain region was measured using a DuoSet ELISA kit (R&D Systems, DY8174-05) according to the manufacturer’s instructions. Samples were read on a TECAN infinite F50 plate reader at 450 nM and 595 nM. Optical correction was performed by subtracting the reading at 595 nM from 450 nM values. The standard curve was plotted using Graphpad prism®, the unknown sample concentrations were calculated by interpolating the absorbances obtained from each sample. Total protein extracted was assessed by Bradford assay (mg protein/mg brain tissue). MMP-9 protein concentration was expressed as pg/mg of total protein.

Perfusion fixed tissue processing

Brains (n = 6 per group) were removed from perfusion fixed animals, post-fixed for 24 h with 4% v/v paraformaldehyde and rinsed in PBS to remove excess fixative. Fixed brain tissue was subsequently dehydrated in graded sucrose solutions (15% and 25% v/v) and stored in 25% sucrose/0.1% sodium azide w/v at 4 °C. Coronal brain sections at 30 μm thickness were cut on a freezing sledge microtome and placed directly onto gelatin-coated slides. Slides were stored at 4 °C in a 0.1% w/v azide/PBS solution prior to staining.

Immunofluorescence staining of MMP-9, microglia and neurons

Brain sections were washed in PBS, blocked in 3% v/v normal goat serum in 0.2% v/v Triton-X/PBS (PBSTx) for 60 min under agitation at room temperature (RT) and incubated with primary antibodies (mouse anti-MMP-9 (1:200, abcam, ab58803), rabbit anti-IBA-1 (1:500, abcam, ab178846) and rabbit anti-Neun (1:200, Novus NBP 1–77686) in 1% v/v normal goat serum/PBSTx for 24 h at RT. Sections were washed in PBSTx and
incubated with secondary antibodies (goat anti-rabbit Alexafluor 488 (1:1000, abcam, ab150116) and goat anti-mouse Alexafluor 594 (1:1000, abcam, ab150077) in 1% v/v normal goat serum in PBSTx for 2 h at RT. Sections were washed in PBS and cover-slipped with DAPI antifade mounting medium and stored in the dark at 4 °C.

Whole slides were scanned on an Olympus VS120 slide scanner (×10 magnification) using DAPI, FITC and CY3 channels. Images were generated in a .vsi format and viewed using the Olympus Olyvia software.

Statistics

Values are expressed as mean ± standard error of the mean in Table 1 or as median and interquartile range in box plots. We used a mixed model experimental design. All data was tested for normality using the Shapiro–Wilk and Kolmogorov–Smirnov tests. All data passed at least one of the two tests with alpha set to 0.05. Physiological parameters and MMP-9 expression across brain regions were analysed using a one-way or two-way ANOVA, corrected for multiple comparisons as appropriate, followed by post-hoc multiple comparisons using Tukey’s test as recommended for data comparing multiple means. A probability of \( p \leq 0.05 \) was considered to be statistically significant. Analysis was performed using GraphPad Prism.

RESULTS

Physiological parameters

Physiological parameters remained stable throughout the surgery, occlusion period and reperfusion period. No significant change in blood pressure, heart rate, arterial pCO2 and pO2, body temperature or blood glucose levels was observed during the 2 h occlusion and reperfusion period (Table 1).

Occlusion of the MCA for 2 h reduced cerebral blood flow (CBF) in the cortical region perfused by the MCA to an average of 47% of pre-occlusion levels (Fig. 2a). This drop in CBF was maintained for the duration of the procedure. Similar reductions in cerebral blood flow were observed in all treatment groups, with significantly reduced cerebral blood flow observed in comparison to sham operated control animals (Fig. 2a).

Following 2 h occlusion, full reperfusion (by complete removal of the occluder), allowed a return in CBF to the affected cortical tissue to 78% of sham CBF levels, which was a significant improvement on the CBF in animals where occlusion was maintained (no reperfusion group), for whom CBF remained at 43% of sham control levels. Partial reperfusion resulted in some re-establishment of improved CBF (53%) (Fig. 2b).

MCA occlusion resulted in a lesion of cortical and striatal tissue (Fig. 3a). Percentage hemispheric lesion volume was affected by reperfusion strategy (\( F_{3,26} = 3.03; \ p \leq 0.05 \), Fig. 3b). Percentage oedema was also affected by reperfusion strategy (\( F_{3,26} = 5.49; \ p \leq 0.01 \), Fig. 3c). Full reperfusion reduced hemispheric lesion volume and oedema to the greatest extent, but
partial reperfusion also reduced hemispheric lesion volume and oedema in comparison to no reperfusion group (Fig. 3b and 3c).

Effect of stroke on hemispheric MMP-9 expression

A significant elevation in MMP-9 expression was observed in four of the five brain regions in the ipsilateral lesioned hemisphere when compared to the contralateral unlesioned hemisphere (Fig. 4). Significantly higher levels of MMP9 were observed in the lesioned frontal cortex, striatum, anterior temporal cortex and posterior temporal cortex, (Table 2 and Fig. 4a-c, e), while there was no significant difference in MMP9 expression in the lesioned and unlesioned hippocampus (Table 2 and Fig. 4d).

Effect of reperfusion strategy on MMP-9 expression

Reperfusion strategy had a significant main effect on MMP9 expression in the lesioned striatum, anterior temporal cortex and posterior temporal cortex (Fig. 4 and Table 2). There was a strong trend towards differences in expression of MMP-9 when comparing reperfusion strategies in the frontal cortex, but no effect in the hippocampus (Table 2). In all brain regions where a hemispheric difference was observed, which was all regions except the hippocampus (Table 2), MMP9 levels were highest in the no reperfusion group (Fig. 4a-c, e). Reperfused tissue had lower MMP9 expression than non-reperfused stroke tissue, with full reperfusion reducing MMP9 levels to a greater extent than partial reperfusion (Fig. 4a-c, e).

In the striatum, MMP-9 expression was significantly higher in all reperfusion strategy groups compared to sham controls. The no reperfusion group showed significantly higher MMP-9 expression compared to all other reperfusion strategies (Fig. 4b).

Discussion

This study was focused on investigating whether different reperfusion strategies had an effect on MMP-9 expression levels in brain tissue following MCA occlusion in a rat model. We observed increased expression of MMP-9 following occlusion in lesioned cortical and striatal brain regions and in the normal appearing ipsilateral frontal cortex. MMP-9 expression, percentage hemispheric lesion volume and oedema were all lower in reperfused brain tissue, most prominently in full reperfusion conditions. Interestingly, the same pattern of effect i.e. levels in non-reperfused tissue > partial reperfused > fully reperfused was observed for MMP-9 expression levels, percentage hemispheric lesion volume and oedema, although only a trend for some parameters. Future work is needed to conclude any possible mechanistic association.

Immunofluorescence was used to confirm ELISA findings and to further investigate the expression pattern of MMP-9 in brain tissue following stroke. The enhanced expression profile in infarcted tissue in the frontal cortex (Fig. 5A), striatum (Fig. 5C and 5D), anterior temporal cortex (Fig. 5B, 5C and 5D) and posterior temporal cortex (Fig. 5E, 5F and 5G) of the lesioned hemisphere was confirmed by immunofluorescence. Fig. 6 shows a magnified image of a region in the anterior temporal cortex expressing high levels of MMP-9. No MMP-9 expression was observed in the contralateral unlesioned hemisphere (Fig. 5A). MMP-9 expression is observed within microvessels and also within nucleated cells in the lesioned hemisphere (Fig. 6C). Clear evidence of microglial activation was observed in the lesioned tissue based on morphological changes in the Iba-1 positive cells (Fig. 6C). Co-localisation of MMP-9 and Neun was also observed suggesting MMP-9 expression by neurons (Fig. 6E).
The BBB regulates permeability through microvascular endothelial tight junctions and the basal lamina (Siflinger-Birnboim et al., 1987). Due to their ability to degrade extracellular matrix and tight junction components, MMPs play a significant role in the BBB breakdown (Turner and Sharp, 2016). Dissolution of the endothelial basal lamina is observed as early as 2 h from the onset of ischaemia, continuing on through reperfusion (Hamann et al., 1995). Early BBB permeability occurs within hours of ischaemia with a second increase in permeability occurring between 24 and 48 h (Rosenberg et al., 1998). MMP-9 levels have been previously shown to be significantly elevated as early as 2 h following cerebral ischaemia (Gasche et al., 1999), and remaining elevated at 24 h and 120 h (Mun-Bryce and Rosenberg, 1998). It is suggested that the early BBB breakdown is caused by a transient increase in MMP-2 levels that precipitates raised MMP-9 levels (Rosenberg et al., 1998; Chang et al., 2003). It is thought that MMP-9 plays an important role in delayed damage (Yang et al., 2007). Non-reversible BBB permeability changes coincide with elevated MMP-9 expression and can result in complete degradation of the basal lamina (Mun-Bryce and Rosenberg, 1998). It has been shown that knockout of MMP-2 had no significant effect on lesion volume following permanent and focal ischaemia, suggesting MMP-2 inhibition does not confer protection against BBB breakdown (Asahi et al., 2001a). Conversely MMP-9 knockout had the effect of reducing degradation of the BBB and conferring protection against focal ischaemia (Asahi et al., 2001b). Administration of a broad spectrum MMP inhibitor BB-94 significantly reduced lesion volume in wild type mice, whereas no further detectable protection was observed in MMP-9 knockout mice following focal ischaemia, further supporting a pivotal role of MMP-9 in BBB breakdown and lesion volume (Asahi et al., 2000).

An association between high levels of MMP-9 and haemorrhagic transformation has previously been demonstrated (Montaner et al., 2001). Elevated levels of MMP-9 in plasma in the acute phase of cerebral infarct evolution is predictive for haemorrhagic transformation in stroke (Castellanos et al., 2003). Increased levels of MMP-9 following the administration of rtPA has previously been shown (Ning et al., 2006), which may be related to the observation that plasmin is involved in the cascade that converts proMMP-9 to its active form (Nagase, 1997). rtPA treatment has been shown to lead to elevated MMP-9 with associated haemorrhagic transformation following embolic focal cerebral ischaemia (Sumii and Lo, 2002).

Clinically, the success of mechanical thrombectomy to treat acute ischaemic stroke has demonstrated that reperfusion as early as possible and to the greatest possible extent produces the best clinical outcomes. A non-significant elevation in serum levels of MMP-9 was previously observed in patients developing severe brain oedema (Moldes et al., 2008). Elevated expression of MMP-9 and MMP-13 have been shown to be involved in lesion growth during the hyperacute phase of stroke (Rosell et al., 2005). During the hyperacute phase of stroke, increased BMP-9 expression correlated to increased Diffusion Weighted Image lesion size in human stroke (Rosell et al., 2005). MMP-9 demonstrated a significant correlation with both the initial and final National Institute of Health Stroke Scale and also with the infarct volume.
Increased rates of haemorrhagic transformation were observed in patients who received thrombolytics, this corresponded to elevated MMP-9 levels (Carbone et al., 2015). It has also been shown that increased levels of MMP-9 are found in areas of haemorrhagic transformation when compared to non-

Fig. 4. Reperfusion strategy had an effect on MMP-9 expression levels. MMP-9 expression levels for sham, full reperfusion, partial reperfusion and no reperfusion in five brain regions, frontal cortex, striatum, anterior temporal cortex, hippocampus and posterior temporal cortex. Procedure was a 2 h MCAO followed by a further 2 h of reperfusion strategy, sham (n = 6), full reperfusion (n = 12), partial reperfusion (n = 6) and no reperfusion (n = 6). Statistical analysis was performed by two-way ANOVA to assess hemispheric and reperfusion strategy effects. Tukey’s multiple comparison test was used to assess significance between the different reperfusion strategies (P ≤ 0.05 *, P ≤ 0.01**, P ≤ 0.001 ***).
haemorrhagic infarct tissue and contralateral tissue (Carbone et al., 2015).

In the present study, using a rat MCA occlusion model of stroke, we also observed that higher MMP-9 expression correlates with increased infarct volume and oedema. We have also demonstrated that partial and full reperfusion resulted in reduced infarct and oedema and also lower expression of MMP-9 in infarcted brain tissue, with full reperfusion having the most pronounced protective effect. The marked similarity in the pattern of the effect of reperfusion strategy on MMP-9 expression and brain injury suggests a possible direct mechanistic involvement, and highlights reducing early increases in MMP-9 expression as a possible neuroprotective therapeutic strategy. Our observation also highlights the rat MCA occlusion model as an ideal model in which to study candidate therapeutics.

Table 2. Statistical analysis by two-way ANOVA for hemispheric and reperfusion strategy effect. Data analysed in five brain regions, frontal cortex, striatum, anterior temporal cortex, hippocampus and posterior temporal cortex. Procedure was a 2 h MCA occlusion followed by a further 2 h of reperfusion strategy.

| Brain Region              | Hemisphere | Reperfusion strategy |
|---------------------------|------------|----------------------|
| Frontal Cortex            | F₁, 52 = 15.51, \( P = 0.0002 \) | F₃, 52 = 2.426, \( P = 0.0759 \) |
| Striatum                  | F₁, 52 = 39.98, \( P ≤ 0.0001 \) | F₃, 52 = 11.53, \( P ≤ 0.0001 \) |
| Anterior Temporal Cortex  | F₁, 52 = 63.85, \( P ≤ 0.0001 \) | F₃, 52 = 10.35, \( P ≤ 0.0001 \) |
| Hippocampus               | F₁, 52 = 1.996, \( P = 0.1637 \) | F₃, 52 = 1.593, \( P = 0.2023 \) |
| Posterior Temporal Cortex | F₁, 52 = 13.34, \( P = 0.0006 \) | F₃, 52 = 2.981, \( P = 0.0397 \) |

Fig 5. Visualisation of area of MMP-9 expression. Immunofluorescence showing microglia (Iba-1, green), MMP-9 (red) and DAPI (blue) expression. Representative series of coronal sections showing regions of heightened MMP-9 expression following 4 h of MCA occlusion of the middle cerebral artery in rat brain (2 h occlusion, followed by a further 2 h of no reperfusion). MMP-9 is highly expressed in the highlighted areas in the anterior temporal cortex (B–D), striatum (C, D), posterior temporal cortex and subcortical tissue (E–G) of the lesioned hemisphere. No overt MMP-9 expression is evident in the normal appearing frontal cortex (A). In the lowest panel, images 5H-5J show indicative differences in MMP-9 expression in the anterior temporal cortex using different reperfusion strategies, sham (5H), full reperfusion (5I) and no reperfusion (5J).
inflammatory response (Kuroiwa et al., 1985). Further investigation of the mechanism of effect of MMP-9 and further assessment of MMP-9 expression by neuronal, glial, infiltrating neutrophils and other immune cell sub-types following stroke is of interest and worthy of further study.

Reperfusion following MCA occlusion resulted in reduced oedema, infarct volume and MMP-9 expression in rat brain tissue. The profiles observed in this study show that full reperfusion after occlusion has beneficial effects, and also even the establishment of partial reperfusion is beneficial in a rat MCA occlusion model. These observations suggest targeting early increases in MMP-9 expression as a possible neuroprotective therapeutic strategy and highlight the rat MCA occlusion model as an ideal model in which to study candidate therapeutics.

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