THE EFFECT OF MECHANICAL REPERFUSION OUTCOME FOLLOWING AN OCCLUSIVE STROKE ON THE REGIONAL EXPRESSION OF MATRIX METALLOPROTEINASE-9 IN RAT BRAIN

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BACKGROUND

- 15 million people suffer from a stroke each year; over 80% of strokes are caused by occlusion.
- Early reperfusion can potentially minimise the degree of brain damage in a stroke caused by occlusion.
- However, reperfusion also can result in serious complications due to abnormal permeability of the blood brain barrier.
- Increased expression of matrix metalloproteinase-9 (MMP-9) in brain tissue is a useful marker of blood brain barrier dysfunction¹.

AIMS

- To study the effect of different mechanical reperfusion outcomes following occlusive stroke on a marker of blood brain barrier dysfunction – matrix metalloproteinase-9 (MMP-9).

METHODOLOGY

- Male Sprague Dawley rats underwent 2 hour middle cerebral artery occlusion (MCAO) (n=6-14 per group).
- Following occlusion, different reperfusion strategies were employed for a further 2 hours; either full, partial or no reperfusion, simulating cerebral blood flow (CBF) restoration that may occur during thrombectomy. In sham-operated animals, filament insertion was omitted.
- Blood pressure, heart rate, blood gases, body temperature and blood glucose were closely monitored and controlled.
- Cerebral blood flow (CBF) was measured using laser Doppler flowmetry.
- Serial coronal brain slices (2mm thick) were incubated in 1% w/v 2, 3, 5-triphenyltetrazolium chloride (TTC) solution at 37°C for 20 minutes to calculate the percentage hemispheric lesion volume (% HLV) and % oedema.
- Brain slices were subsequently dissected and the expression of MMP-9 in the frontal cortex, striatum, anterior temporal cortex, posterior temporal cortex and hippocampus was assessed by ELISA (DuoSet, R&D Systems).

RESULTS

- Blood pressure, heart rate, blood gases, body temperature and blood glucose were all stable and within normal physiological limits throughout.

	Sham			Full reperfusion			Partial reperfusion			No reperfusion		
	Pre	During	Post	Pre	During	Post	Pre	During	Post	Pre	During	Post
Blood pressure mmHg	69 ± 15	65 ± 14	61 ± 12	80 ± 14	70 ± 13	64 ± 13	67 ± 13	70 ± 12	67 ± 20	77 ± 15	64 ± 10	63 ± 15
Heart rate bpm	392 ± 37	372 ± 46	384 ± 47	403 ± 66	371 ± 48	391 ± 65	393 ± 52	398 ± 63	389 ± 55	370 ± 44	375 ± 18	385 ± 55
Arterial pCO ₂ mmHg	29 ± 6	33 ± 7	35 ± 6	32 ± 7	35 ± 5	37 ± 9	34 ± 7	37 ± 7	39 ± 13	30 ± 5	33 ± 3	34 ± 4
Arterial pO ₂ mmHg	136 ± 43	116 ± 40	119 ± 33	147 ± 23	140 ± 33	135 ± 30	135 ± 31	122 ± 19	116 ± 33	142 ± 47	126 ± 38	120 ± 19
Body temp °C	37.1 ± 0.6	37.5 ± 0.4	37.4 ± 0.5	37.3 ± 0.5	37.3 ± 0.5	37.1 ± 0.6	37.3 ± 0.3	37.7 ± 0.1	37.7 ± 0.1	37.3 ± 0.5	37.7 ± 0.2	37.7 ± 0.2
Blood glucose mmol/L	10.7 ± 3.4			12.3 ± 4.1			9.4 ± 2.7			11.8 ± 2.6		

Table 1: Physiological parameters. Statistical analysis by 2-way ANOVA, no statistically significant changes were observed

REFERENCES

1. Heo, J. H., et al. (1999) Matrix metalloproteinases increase very early during experimental focal cerebral ischemia *Journal of Cerebral Blood Flow & Metabolism* **19** (6): 624-633

RESULTS

- MCAO caused a drop in CBF to 50% of pre-occlusion level ($F(3,37)=10.57$; $p<0.001$) (Fig 1). Reperfusion approach had a significant effect on CBF ($F(2,27)=5.12$; $p<0.05$); 78%, 53% and 43% of sham levels for full, partial and no reperfusion groups respectively (Fig 2).

Fig 1

Fig 2

Fig 1 and 2: Blood flow changes during surgery. Statistical analysis by 1-way ANOVA with Tukey's multiple comparison test

- Occlusion resulted in significant infarction ($F(3,26)=3.03$; $p<0.05$), most prominent in non-reperused animals (Fig 3), and also significant oedema ($F(3,26)=5.49$; $p<0.01$) (Fig 4).

Fig 3

Fig 4

Fig 3 and 4: % HLV and oedema. Statistical analysis by 1-way ANOVA with Tukey's multiple comparison test

- Occlusion resulted in increased MMP-9 expression in the lesioned hemisphere in the frontal cortex ($F(1,52)=15.51$; $p<0.001$) (Fig 5), striatum ($F(1,52)=39.98$; $p<0.001$) (Fig 6), anterior temporal cortex ($F(1,52)=63.85$; $p<0.001$) (Fig 7) and posterior temporal cortex ($F(1,52)=13.34$; $p<0.001$) (Fig 8), but not the hippocampus ($F(1,52)=2.00$; ns) (Fig 9).
- MMP-9 expression was higher in animals not reperused than those fully or partially reperused, particularly in the striatum ($F(3,52)=11.53$; $p<0.001$) (Fig 6) and anterior temporal cortex ($F(3,52)=10.35$; $p<0.001$) (Fig 7).

Fig 5

Fig 6

Fig 7

Fig 8

Fig 9

Fig 5-9: MMP-9 expression in brain regions in the lesioned and unlesioned hemispheres. Statistical analysis by 2-way ANOVA with Tukey's multiple comparison test * $p<0.05$ vs ipsilateral sham; t $p<0.05$ vs ipsilateral no reperfusion

CONCLUSION

- Permanent occlusion resulted in greater infarct, oedema and MMP-9 expression than full or partial reperfusion, supporting the clinical benefits of early thrombectomy and indicating the benefit of even partial reperfusion.

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