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RESEARCH LETTER

Time-dependent biochemical response after middle cerebral artery occlusion in rats: A pilot study

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Ischaemic stroke outcome depends largely on how quickly and severely brain tissue is injured before blood flow is restored [1]. Because occlusion duration is one of the few variables clinicians can influence through faster recanalisation, understanding how it shapes the biology of injured tissue is central to defining a realistic treatment window and identifying biomarkers of urgency. Rodent's middle cerebral artery occlusion, with varying occlusion duration, allows comparison of biochemical and histological responses across ischaemic severities [2, 3]. However, whether occlusion duration differentially affects oxidative-stress, inflammatory, and neurovascular-repair markers within the same 24-hour post-reperfusion window, and whether such changes precedes visible structural injury, remain unclear. This pilot study, consistent with the 3Rs principle of Reduction, screened 20-, 30-, and 60-minute occlusion durations to identify which produces a reproducible, well-tolerated injury profile suitable for an adequately powered main study. Group size followed the 3Rs principle of Reduction rather than a power calculation.

Six adult male Wistar rats (250–300 g) were randomly allocated to three groups (2 in each group), each undergoing right middle cerebral artery oclusion for a fixed duration of 20, 30, or 60 minutes using the intraluminal filament model. Rats were anaesthetised with isoflurane and maintained on a homeothermic pad (37±0.5°C). A silicone-coated monofilament was advanced via the external carotid artery to occlude the MCA origin, confirmed by a regional cerebral blood flow reduction of at least 80%. Reperfusion was achieved by filament withdrawal. Investigators performing assays and histology were blinded. All six rats survived to the 24-hour endpoint, when tissue was collected under terminal anaesthesia. Ipsilateral cortical/striatal tissue was homogenised in radioimmuno-precipitation assay buffer, and total protein was quantified by bicinchoninic acid assay. GSH-Px and superoxide dismutase activity and IL-1β and IL-6 concentrations were measured using activity assays/ELISA (normalised to total protein), and NRP-1 expression was assessed by Western blot (normalised to β–actin). Paraffin-embedded coronal sections were stained with haematoxylin and eosin and scored (20×, three fields/section) for percentage hemisphere involvement (oedema-corrected), pyknosis, vacuolation, and gliosis (0–4 scale).

ANOVA was done to find out overall differences among groups; then pairwise comparisons (unpaired *t* test) were Bonferroni-corrected for three comparisons per marker (adjusted threshold $P<0.017$).

Key messages

In a rat model of transient occlusion of the middle cerebral artery for 20, 30, or 60 minutes; the 60-minute duration produced the most pronounced, statistically significant shifts in oxidative-stress, inflammatory, and vascular-repair markers, identifying it as the ischaemic duration most likely to reveal meaningful biological change in future studies.

Table 1 Mean (standard deviation) of biochemical marker levels in ipsilateral tissue in three groups (two rats per group) with middle cerebral artery occlusion

Marker	Group 1 (20 min)	Group 2 (30 min)	Group 3 (60 min)	*P*
GSH-Px (U/mg protein)	315.4 (6.9)	335.5 (9.4)	407.3 (3.8)[a]	<0.01
SOD (U/mg protein)	79.3 (1.5)	123.4 (3.5)[b]	88.3 (2.6)	<0.001
IL-1β (pg/mg protein)	122.1 (4.4)	115.4 (4.4)	61.0 (1.4)[a]	<0.001
IL-6 (pg/mg protein)	9.6 (0.3)	10.1 (0.0)	12.0 (0.3)[c]	<0.01
NRP-1 (relative expression)	398.7 (11.2)	403.2 (10.6)	502.6 (3.7)[a]	<0.01

GSH-Px indicates glutathione peroxidase; SOD, superoxide dismutase; IL, interleukin; NRP-1, Neuropilin-1.

[a]Group 1 vs Group 3 and Group 2 vs Group 3 both significant (*P*<0.05, Bonferroni-corrected).

[b]Group 2 significantly higher than both Group 1 and Group 3 (*P*<0.05). [c]Group 2 vs Group 3 significant (*P* <0.05).

Haematoxylin and eosia staining showed comparable mild ischaemic changes across all three groups, with no group showing qualitatively more severe histological injury than another. Biochemical findings are summarised in Table 1. GSH-Px and NRP-1 were significantly higher, and IL-1β significantly lower, in the 60-minute group than in shorter-duration groups. Superoxide dismutase showed a distinct, non-monotonic pattern, peaking at 30 minutes rather than changing progressively with duration. IL-6 was significantly higher at 60 minutes than at 30 minutes, though its difference from 20 minutes narrowly missed significance after correction. As each group comprised a separate pair of animals rather than repeated measurements, these results describe differences between independent occlusion-durations.

These findings suggest occlusion duration differentially engages oxidative-stress, inflammatory, and vascular-repair pathways before injury is apparent on routine histology, consistent with the broader molecular cascade described for ischaemic stroke [4].

The rise in GSH-Px and NRP-1 alongside the fall in IL-1β at 60 minutes may reflect a compensatory antioxidant and pro-angiogenic response intensifying with longer ischaemic exposure, in line with neuropilin-1's established role in regulating vascular permeability during ischaemic injury [5]. The concurrent rise in IL-6 at 60 minutes may further reflect duration-dependent inflammatory signaling as previously implicated in stroke severity [6]. Nonetheless, the non-monotonic superoxide dismutase peak at 30 minutes could mark an intermediate threshold at which enzymatic defences are maximally recruited before being overwhelmed, consistent with the engagement of both injury and endogenous protective pathways described in the wider literature [7, 8].

Because biochemical change appears to precede structural injury, such markers could eventually help identify tissue still amenable to intervention before infarction is established. However, this pilot cannot establish that on its own. Given the small sample (2 per group), absence of control group, single timepoint, and non-standardised biochemical assay protocols across the groups, these results need confirmation in an adequately powered study.

As a screening pilot, these results are best used to inform the design of the main study rather than as a stand-alone conclusion. The 60-minute duration produced the most consistent and significant shifts across GSH-Px, NRP-1, and IL-1β without a corresponding increase in histological severity, making it a reasonable candidate duration to carry forward. Superoxide dismutase's distinct and non-monotonic response in the 30-minute group is also worth retaining as a comparison arm.

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Author contributions

Conception and design, or design of the research; or the acquisition, analysis, or interpretation of data: AP, PS, LP. *Drafting the manuscript or revising it critically for important intellectual content:* YU, DP, AP, Ak.T. *Final approval of the version to be published:* PS, LP. *Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved:* AP.

Conflict of interest

We do not have any conflict of interest.

Data availability statement

Data supporting the findings of the study are available from the corresponding author upon reasonable request.

AI disclosure

During the preparation of this manuscript, the author(s) used AI Tools in order to refine the academic language.

Supplementary file

None

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