

Short report

# Do leukocytes have a role in the cerebral no-reflow phenomenon?

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**SUMMARY** The possible role of leukocytes in the cerebral microcirculation following ischaemia was assessed in the gerbil. The no-reflow phenomenon seen after 30 minutes of severe bilateral hemispheric ischaemia during hypotensive reperfusion was compared in control animals and in a group made leukopenic by pretreatment with cyclophosphamide. Neither the incidence nor the severity of the no-reflow phenomenon differed between the two groups. The evidence from this study casts doubt on the hypothesis that leukocyte plugging plays a major role in the cerebral microcirculation's response to ischaemia.

There is increasing interest in the rheological properties of leukocytes.<sup>1</sup> The peripheral blood leukocyte count has been shown to be a predictor of myocardial infarction, and stroke,<sup>2-6</sup> and leukocyte counts correlate with the size of myocardial infarcts.<sup>7</sup> A poor prognosis after myocardial infarction,<sup>8,9</sup> and stroke<sup>10</sup> is associated with a high leukocyte count. In experimental models of myocardial infarction, reducing the leukocyte count reduces infarct size.<sup>11</sup> The role of leukocytes in the cerebral circulation has received less attention,<sup>12</sup> but cellular plugging has been suggested as a contributory factor in the genesis of the "no-reflow" phenomenon.<sup>13</sup> This is characterised by patchy poor perfusion after arrest of the cerebral circulation, if reperfusion is initially at an untreated low perfusion pressure.<sup>14</sup>

We sought to investigate the role of leukocytes in the cerebral circulation by studying the effect of lowering the peripheral blood leukocyte count on the development of the no-reflow phenomenon in the gerbil brain.

## Methods

The no-reflow phenomenon was produced in the gerbil by 30 minutes of severe bilateral hemispheric ischaemia followed

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by reperfusion without blood pressure support. Adult gerbils of either sex (60-80 g) were anaesthetised with intraperitoneal pentobarbitone 60 mg/kg (Sagatal May and Baker Ltd). Through a midline cervical incision a tracheostomy was performed and both common carotid arteries were isolated with 5-0 silk sutures. A cannula was placed in the left femoral artery for blood sampling and continuous blood pressure monitoring. The left femoral vein was cannulated for injection of India ink. Arterial blood (0.1 ml) was removed for measurement of leukocyte count and haematocrit and in some of the animals a platelet count.<sup>15</sup> Scoville-Lewis aneurysm clips were then placed on both carotid arteries for 30 minutes. Ten minutes after their removal, an intravenous injection of 1 ml of an isotonic filtered suspension of India ink was given over the course of 30 seconds. (Pulmonary filtration further removes larger particles and the cerebral microcirculation is thereby fully perfused.) This technique also ensures that the intravascular marker circulates at the prevailing arterial blood pressure. After a further minute of reperfusion with India ink the animal was decapitated and the brain removed and fixed in 10% neutral formal saline. Coronal (1 mm) sections were dehydrated in alcohol and cleared in oil of Wintergreen (methyl salicylate) for subsequent visual inspection. Brain slices were reviewed without access to other data and the degree of capillary filling recorded as normal or abnormal. Abnormal patterns were further divided into four grades.

*Grade 1* Minimal abnormality

*Grade 2* Mild abnormality

*Grade 3* Moderate abnormality

Occasional cortical radial capillaries unfilled.  
Small areas of cortical &/or subcortical non-filling.  
Confluent areas of cortical and subcortical non-filling.

Table 1 Blood pressure levels (mm Hg + sd) to nearest 5 mm

Group	Pre-occl.	During occl.	During reperfusion
Cyclo (n = 15)	55 + 15	80 + 20	40 + 15
Saline (n = 17)	65 + 10	80 + 20	50 + 15

**Grade 4 Severe abnormality** Extensive areas of poor perfusion including cortex subcortex & basal ganglia.

Two groups of gerbils were subjected to severe bilateral hemispheric ischaemia in this manner. The first group received four consecutive daily intraperitoneal injections of cyclophosphamide 50 mg/kg (Farmitalia, Carlo Erba Ltd) in order to suppress their peripheral blood leukocyte count. Control animals received the same number of injections of normal saline. At the time of each injection an additional intraperitoneal injection of "Mesna" (sodium 2 mercaptoethanesulphonate WB Pharmaceuticals Ltd) 50 mg/kg was given to limit the nephrotoxic effects of cyclophosphamide. Controls also received injections of "Mesna". All animals were anaesthetised for surgery the day after the last injections.

**Results**

In two animals the India ink perfusion was unsuccessful for technical reasons, and in five others the blood pressure did not fall below their starting blood pressure on clip removal. Under these circumstances the no-reflow phenomenon does not develop,<sup>14</sup> so these animals were excluded. Blood pressure changes were comparable in the two groups with the characteristic response to bilateral carotid clipping of a sustained increase in pressure during occlusion and a fall after clip removal (table 1). Cyclophosphamide injections in 15 animals lowered the whole blood leukocyte count by 85% with much smaller changes in platelet count and haematocrit (table 2). Seventeen controls completed the protocol.

The no-reflow phenomenon was observed in 10 of the cyclophosphamide treated animals and 13 of the controls (table 3). This difference is not significant, and the ratings of severity by the blinded observer showed no difference between the two groups.

Table 2 Haematological values (mean + sd)

	Cyclophosphamide (15)	Controls (17)
WBC 10 <sup>9</sup> /l	0.89 + 0.61	6.13 + 4.12‡
Platelets 10 <sup>11</sup> /l	4.49 + 1.38 (n = 5)	6.17 + 1.66* (n = 9)
HCT%	34.5 + 3.5	39.5 + 5.0†

\*NS.  
† p < 0.01.  
‡ p < 0.001.

Table 3 Incidence and severity of no-reflow

Grade	Cyclophosphamide	Control
Normal	5	4
1	1	1
2	2	4
3	4	5
4	3	3
Total	15	17

**Discussion**

This study has failed to reveal any evidence that a grossly reduced leukocyte count reduces the incidence or severity of the no-reflow phenomenon in the gerbil brain subjected to 30 minutes of severe bilateral hemispheric ischaemia. The marrow suppression produced by the cyclophosphamide also reduced platelet numbers and haematocrit though to a smaller extent. These effects might also have been expected to improve perfusion as haemodilution can reduce the no-reflow phenomenon,<sup>13</sup> and the presence of platelet aggregates is believed to be a contributory factor in obstructing the lumen of small vessels for example after myocardial infarction.<sup>16</sup>

When Fischer and Ames first described the no-reflow phenomenon they saw it as a critical factor in the brain's peculiar sensitivity to ischaemia.<sup>13</sup> They thought that the mechanism underlying the impaired perfusion of the brain had to do with endothelial changes or glial cell swelling. Subsequently cellular plugging of capillaries has been considered at least as important with evidence of increased viscosity in stagnant blood, platelet and red cell aggregation, and leukocyte plug formation.<sup>13,17</sup> The present results suggest that leukocyte numbers are not critical to development of the no-reflow phenomenon. This makes a rheological explanation for the epidemiological evidence of leukocyte involvement in cerebrovascular disease somewhat less likely.

There is of course a chicken and egg argument over the finding of an increased stroke mortality in those with a high leukocyte count,<sup>10</sup> or with abnormal leukocyte rheology after acute stroke.<sup>18,19</sup> The blood changes may be part of the stress response which also elevates fibrinogen and erythrocyte sedimentation rate.<sup>20</sup> Only interventional studies could determine whether the peripheral leukocytosis is pathologically significant in acute stroke victims. The present study lends little support to such a proposal.

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