Transient Appearance of “No-Reflow” Phenomenon in Mongolian Gerbils

Umeo Ito, M.D., Kikuo Ohno, M.D., Takekane Yamaguchi, M.D., Hiroki Tomita, M.D., Yutaka Inaba, M.D., and Masatoshi Kashima

SUMMARY We investigated the existence of the “no-reflow” phenomenon in focal cerebral ischemia. Regional cerebral blood flow was studied in Mongolian gerbils perfused with a carbon-black particle suspension after cerebral ischemia prior to decapitation and compared with 14C-antipyrine autoradiographic images. The correlation between the occurrence of the “no-reflow” phenomenon and systemic arterial blood pressure change was also examined. We found that the phenomenon was transient in character and that its manifestation was related to the transient fall in arterial blood pressure observed immediately after clip release and with stagnation of venous blood flow. The phenomenon disappeared in animals in which the arterial blood pressure was artificially increased after clip release.

Materials and Methods

Two groups of adult Mongolian gerbils of either sex were used. In the first group, the common carotid artery was occluded bilaterally under light ether anesthesia, using Scovill's aneurysmal clips. The second group consisted of ischemia-sensitive gerbils which were selected as described previously; in this group unilateral occlusion of the left common carotid artery was performed.

1. Assessment of “No-Reflow” Phenomenon by Carbon-Black Perfusion

Cerebral blood flow was restored 30 sec to 15 min after 5, 15, 30, or 60 min bilateral, or 30 min, 1, 3 or 6 h unilateral occlusion. The animals were lightly anesthetized with ether and, 30 sec prior to decapitation, 1 ml of a carbon-black suspension for biological use (Pelikan Werke, W. Germany) was injected into the femoral vein. After formalin fixation of the whole brain, it was cut into 3 coronal blocks at the level of the optic chiasm and infundibulum. The patency of the vascular tree was confirmed on the cut surfaces under a dissecting microscope as well as by histology. The brain was considered positive for the “no-reflow” phenomenon when there was more than one non-perfused area measuring more than 2 mm in diameter.

2. Morphological Assessment of rCBF by 14C-Antipyrine Autoradiography and Benzidine Preparations

Cerebral blood flow was restored 30 sec to 1 h after 1 h bilateral, or 6 h unilateral occlusion. The animals were fixed with 10% formalin and the brains cut into 3 coronal blocks at the level of the optic chiasm and infundibulum. The patency of the vascular tree was confirmed on the cut surfaces under a dissecting microscope as well as by histology. The brain was considered positive for the “no-reflow” phenomenon when there was more than one non-perfused area measuring more than 2 mm in diameter.

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were lightly anesthetized with ether and during the 60 sec preceding decapitation, 7.5 \mu Ci of \(^{14}C\)-antipyrine (36.5 \mu Ci/mM) dissolved in 1 ml saline, were injected at constant speed into the femoral vein. The posterior half of the brain was frozen immediately with dry ice, 30 \mu m thick sections were cut, brought into contact with Kodak medical x-ray film (SB54) and kept dry in the dark for 2 weeks at 4°C. As rCBF is almost parallel to autoradiographical image density, we assessed the ischemic and hyperemic portions of the post-ischemic hemisphere by comparing them with the autoradiographical image density of the cerebral hemisphere of control animals.

The anterior half of the brain was fixed in a buffered paraformaldehyde solution (pH 7.3). Frozen sections, 30 \mu m in thickness, were obtained from the side facing the posterior blocks and benzidine-stained.

3. Effect of Arterial Blood Pressure Changes on the "No-Reflow" Phenomenon

Five animals were subjected to 1 h bilateral or 6 h unilateral occlusion. To monitor the systemic arterial blood pressure on a Statham transducer during the 15 min following blood flow restoration, the animals were lightly anesthetized with ether and a PE-10 plastic catheter (Clay-Adams, USA) was inserted into the abdominal aorta through the femoral artery, using an operating microscope.

4. Effect of Sympathomimetics on the "No-Reflow" Phenomenon

Animals with bilaterally and unilaterally occluded carotid arteries were i.v. injected with 0.5 gamma/100 g of epinephrine, norepinephrine or isoproterenol 30 sec before clip release and 2 min before sacrifice. The mean arterial blood pressure (MABP) was monitored before and for 5 min after blood flow restoration. Then each animal was given an injection of 1 ml of carbon-black suspension into the femoral vein 30 sec prior to sacrifice. The number of animals exhibiting the "no-reflow" phenomenon was determined 5 min after blood flow restoration.

Results

An independent study revealed that in 5 gerbils with carotid arteries bilaterally clipped for 1 h, the average Paco\(_2\) was 38.4 ± 4.0 mm Hg, Paco\(_2\) was 114.3 ± 7.8 mm Hg and pH was 7.347 ± 0.016. In 5 gerbils with the carotid artery unilaterally clipped for 6 h, the corresponding values were 37.2 ± 3.3 mm Hg, 114.0 ± 10.1 mm Hg and 7.39 ± 0.013.

TABLE 1  Incidence of "No-Reflow" Phenomenon as Assessed by Carbon-Black Perfusision*  

<table>
<thead>
<tr>
<th>Carotid Occlusion</th>
<th>Time after Restoration of Blood Flow</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>30 sec</td>
</tr>
<tr>
<td>Bilateral</td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>0/5†</td>
</tr>
<tr>
<td>15 min</td>
<td>4/4</td>
</tr>
<tr>
<td>30 min</td>
<td>5/5</td>
</tr>
<tr>
<td>1 hr</td>
<td>5/5</td>
</tr>
<tr>
<td>Unilateral</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>0/5</td>
</tr>
<tr>
<td>1 hr</td>
<td>1/5</td>
</tr>
<tr>
<td>3 hr</td>
<td>7/7</td>
</tr>
<tr>
<td>6 hr</td>
<td>5/5</td>
</tr>
</tbody>
</table>

*This was determined in relation to duration of bilateral or unilateral carotid occlusion and time after blood flow restoration.

†Number of animals exhibiting the "no-reflow" phenomenon/number of animals examined.

1. Appearance of the "No-Reflow" Phenomenon Assessed by Carbon-Black Perfusion

The "no-reflow" phenomenon was visualized as irregularly scattered patchy areas with incomplete perfusion (fig. 1a).

The phenomenon was not present in any of the animals subjected to bilateral 5 min occlusion (table 1). On the other hand, in all 5 gerbils with bilateral

FIGURE 1. "No-reflow" phenomenon as demonstrated by carbon-black perfusion. Patchy non-perfused areas at 30 sec (a) and well-perfused brain at 15 min (b) after blood flow restoration following 1 h bilateral carotid occlusion.
occlusion for 1 h, it was noted 30 sec after blood flow restoration (fig. 1a). It was not present in any of the animals examined at 15 min after blood flow restoration (fig. 1b). At 30 sec after clip release, the “no-reflow” phenomenon was not present in animals with unilateral occlusion for 30 min, but it was noted in all gerbils that had been subjected to unilateral occlusion for 6 h and in this group the phenomenon was not present at 10 min after clip release.

2. Morphological Assessment of rCBF by "C-antipyrine Autoradiography and Benzidine Preparations

In the post-ischemic hemispheres, ischemic and hyperemic areas were recognized by a density difference on the \(^{14}\)C-antipyrine autoradiographic images (fig. 2a, b).

After blood flow restoration in the 1 h bilateral and 6 h unilateral occlusion groups, the incidence of hyperemic portions increased and that of ischemic portions decreased with passage of time after blood flow restoration (table 2).

The benzidine preparations which demonstrated red blood corpuscles filling vascular beds showed marked dilatation of the blood vessels on the clipped side. Animals in which ischemic injury of the brain parenchyma was severe showed markedly engorged large veins surrounding areas which appeared pale due to poor capillary filling. These findings indicate that the blood had become stagnant primarily on the venous side of the cerebral circulation (fig. 3).

3. Arterial Blood Pressure Change and “No-Reflow” Phenomenon

In 5 gerbils with bilateral occlusion for 1 h, the average MABP just prior to clip release was 69.2 mm Hg, it dropped to 43.8 mm Hg within 30 sec of release and increased gradually to 68.0 mm Hg by 15 min. In 5 gerbils unilaterally occluded for 6 h, a similar pattern of precipitous blood pressure drop just after clip release and a gradual increase thereafter was noted (table 3).

4. Effect of Sympathomimetics on “No-Reflow” Phenomenon

In animals after 1 h bilateral and 6 h unilateral occlusion, epinephrine and norepinephrine were effective in maintaining MABP above the control level and in reducing the incidence of the “no-reflow” phenomenon (table 4). On the other hand, isoproterenol, a beta-stimulant, slightly decreased MABP and was not effective in reducing the incidence of the “no-reflow” phenomenon.

Discussion

The number of animals with ischemic areas as identified by autoradiography (table 2) and the number of those with the “no-reflow” phenomenon as assessed by carbon-black perfusion (table 1) were in good agreement. Taken into account were the duration and severity of the ischemic insult and the passage of time between clip release and disappearance of the “no-reflow” phenomenon.

The duration of the “no-reflow” phenomenon in our focal ischemia model depended on the duration of ischemia, but it was transitory when it occurred.

![Figure 2. \(^{14}\)C-antipyrine autoradiograms. Patchy ischemic portions at 30 sec (a) and diffuse hyperemic brain at 30 min (b) after blood flow restoration following 1 h bilateral carotid occlusion.](http://stroke.ahajournals.org/)

![Figure 3. 6-h ischemic left cerebral hemisphere shows strongly engorged venous vessels surrounding areas which are pale due to poor blood filling of the capillaries (Lephene-Pickworth benzidine stain).](http://stroke.ahajournals.org/)
The phenomenon, as demonstrated by carbon-black perfusion, did not appear when the ischemic insult was of short duration and it was not present 15 min after clip release even after 1 h of bilateral or 6 h of unilateral carotid artery occlusion. The duration of the phenomenon was longer following bilateral than unilateral occlusion (table 1), possibly due to the greater severity of the ischemic insult and the more pronounced drop in blood pressure immediately upon clip release in the bilaterally occluded animals (table 3). 14C-antipyrine autoradiography showed rCBF to have recovered after 30 min in both bilaterally and unilaterally occluded animals, and the occurrence of postischemic reactive hyperemia (table 2). 11, 12

The duration of the "no-reflow" phenomenon closely correlates with a change in systemic arterial blood pressure after clip release. 8, 10 In both 1 h bilateral and 6 h unilateral occlusion experiments, clip release produced a rapid and marked drop in systemic arterial blood pressure which, however, recovered within 15 min to the pre-release level. The number of animals exhibiting the "no-reflow" phenomenon also decreased as time after clip release increased. By 15 min post-release, none of the animals exhibited the "no-reflow" phenomenon (table 1).

Of the sympathomimetics examined, epinephrine proved to be the most effective in reducing the incidence of the "no-reflow" phenomenon. Furthermore, it prevented the blood pressure drop that usually occurs immediately following clip release (table 4). 9, 10

Our findings indicate that the transient "no-reflow" phenomenon in the present ischemia model is not due to organic changes as has been suggested, 8, 13, 14 but rather to functional changes.

We propose the following hypothesis to explain the functional changes which result in the appearance of the "no-reflow" phenomenon. Occlusion of the carotid artery reduces rCBF and the perfusion pressure to near zero levels. This results in a decrease of the diameter of the resistance vessels as there is a marked decrease in the pressure gradients along the arteries, arterioles and capillaries. As demonstrated by our benzidine preparations, 9, 13 blood stagnates on the venous side of the cerebral circulation, and hemoconcentration may occur. 13-18 Due to the systemic blood pressure drop, there is no recirculation against the existing vascular resistance. 13-21

Systemic arterial blood pressure changes alone, however, do not explain the fact that the "no-reflow" phenomenon lasted longer in animals exposed longer to ischemia (table 1). This may be related to increased vascular resistance due to perivascular edema, 24 hemoconcentration and venous stagnation.

In the complete ischemia model most of the vascular lumens may be collapsed during ischemia. 11, 3 In the present focal ischemia model rCBF, while reduced to the oligemic state, did not cease completely (Ohno K, Ito U, Yamaguchi T, Takei H, Tomita H, Inaba Y: Regional cerebral blood flow study with 'H-nicotine in Mongolian gerbils with different stroke indices. In preparation). This indicates that most of the vessels remained open although there may be some narrowing during carotid artery occlusion. In the complete ischemia model, upon clip release, the blood can flow only through those vessels which, by chance, may have remained open during the ischemic insult, which may explain why the "no-reflow" phenomenon is extensive and severe in the complete ischemia model.

Electronmicroscopic evidence of organic obstruction and narrowing of the capillaries as a cause of the "no-reflow" phenomenon in complete 8, 2 and focal ischemia 13, 14 has been presented. Our previous electronmicroscopic study 25 showed no organic changes of the vascular endothelial cells. 25, 24 Although there are several controversial reports, 13, 14, 26 we found that in focal ischemia, the "no-reflow" phenomenon is transient and associated with the arterial blood pressure drop that occurs immediately after clip release and with stagnation of the venous blood flow. Furthermore, the phenomenon could be abolished by increasing the arterial blood pressure, 10, 13, 26 despite the fact that tissue injury and blood-brain barrier change progressed after restoration of the blood flow to the ischemic brain. 26, 27

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**Table 4** The Effect of Sympathomimetics on the Incidence of the "No-Reflow" Phenomenon

<table>
<thead>
<tr>
<th>Carotid Occlusion</th>
<th>Treatment</th>
<th>Total No. of Animals</th>
<th>Animals with &quot;No-Reflow&quot;</th>
<th>Average Mean Arterial Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before 30 s 5 min After Clip Release</td>
</tr>
<tr>
<td>1-h bilateral</td>
<td>No treatment</td>
<td>5</td>
<td>4</td>
<td>69.2 ± 2.0 43.8 ± 0.5 52.5 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>4</td>
<td>4</td>
<td>158.8 ± 5.2 102.5 ± 8.5 76.3 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Norepinephrine</td>
<td>4</td>
<td>1</td>
<td>130.3 ± 5.5 88.8 ± 1.3 77.5 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>4</td>
<td>4</td>
<td>61.3 ± 2.4 46.3 ± 2.4 60.0 ± 2.9</td>
</tr>
<tr>
<td>6-h unilateral</td>
<td>No treatment</td>
<td>7</td>
<td>5</td>
<td>77.0 ± 1.2 60.0 ± 2.6 67.0 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>4</td>
<td>0</td>
<td>127.5 ± 3.2 125.0 ± 2.0 78.8 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>Norepinephrine</td>
<td>4</td>
<td>2</td>
<td>130.0 ± 8.4 106.3 ± 7.5 80.8 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>Isoproterenol</td>
<td>4</td>
<td>3</td>
<td>52.5 ± 6.3 40.0 ± 6.1 51.3 ± 12.1</td>
</tr>
</tbody>
</table>

The average mean arterial blood pressure of 8 control animals was 86.0 ± 3.1 mm Hg (± se). *6 min after clip release.
References

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