Establishment of a Cutaneous Flap Animal Model to Study Platelet and Leukocyte Dynamics After Ischemia-Reperfusion Injury

Timothy S. Lian, MD; Andrew Compton, MD; Rebecca Bowen, MD

Objectives: To study a cutaneous flap in an animal model for platelet and leukocyte dynamics after ischemia-reperfusion injury and to explain how such a model is relevant to the understanding of reconstructive flaps in a clinical setting.

Methods: Cutaneous flaps based on the inferior epigastric artery were raised on C57BL/6 mice and were subjected to various periods of ischemia followed by reperfusion. We used intravital microscopy to observe and characterize platelet and leukocyte interactions within the microvasculature.

Results: Platelet and leukocyte adherence to the microvasculature was greater after a longer reperfusion period in contrast to the adherence pattern seen after a shorter reperfusion period. Leukocyte rolling activity occurred at a greater rate after longer ischemia and shorter reperfusion periods, whereas the rate of platelet salivation occurred after shorter ischemia and longer reperfusion periods.

Conclusions: With the establishment of an animal model of cutaneous flaps to study cellular dynamics within the microvasculature after ischemia-reperfusion injury, further investigation into the cellular and molecular characteristics of such injury and the analysis of pharmacological interventions is warranted.


Reconstructive surgery of the head and neck frequently involves use of various flaps and grafts. Successful reconstructions rely on the survival of these transferred tissues. Flaps and free grafts that have been subjected to ischemia are at risk for partial or total failure after reperfusion. Platelet activation and leukocyte recruitment have been implicated in ischemia-reperfusion injury in the brain and the gastrointestinal tract.\(^1\)\(^,\)\(^2\) Damage to the microvasculature is also thought to play a role in tissue injury.\(^3\)\(^,\)\(^4\) Analysis of the microvasculature may provide insight into cellular and molecular processes that occur in cutaneous flaps that have been subject to ischemia followed by reperfusion. We sought to analyze platelet and leukocyte dynamics with intravital microscopy in ischemia-reperfusion injury using an animal model of cutaneous flaps. We used fluorescence and intravital microscopy to characterize platelet and leukocyte dynamics within the microvasculature of cutaneous flaps subjected to ischemia-reperfusion injury. We analyzed the influence of varying durations of ischemia followed by reperfusion on the activation of leukocytes and platelet adhesion in the postcapillary venules of pedicled cutaneous flaps in a mouse model. If applied to ischemic flaps in an animal model, pharmacological and other interventions can be studied with respect to cellular activity in the microvasculature of ischemic tissues. Such studies may aid in the analysis of which interventions might be beneficial to flap survival in a clinical setting.

Methods: All experiments involving animals were approved by the Animal Care and Use Committee at the Louisiana State University Health Sciences Center Shreveport.

We used C57BL/6 mice for the studies. After the mice were anesthetized with ketamine hydrochloride and xylazine hydrochloride, we raised a fasciocutaneous pedicled flap based on an inferior epigastric vascular pedicle (Figure 1). Flaps were then subjected to 0 (sham), 15, 30, 60, or 90 minutes of no-flow...
ischemia by clamping the inferior epigastric vessels, followed by 30 minutes or 4 hours of reperfusion. We used 6 to 8 mice for each period of ischemia and reperfusion. The sham ischemia mice were used as time-matched control subjects in that the vascular pedicle was not clamped, thus allowing for no ischemia. Cutaneous flaps were raised on a total of 71 mice. At the conclusion of each ischemic period, the clamp was removed and the flaps were observed with intravital microscopy using a fluorescein isothiocyanate filter after 30 minutes or 4 hours of perfusion. Leukocyte and platelet-endothelial cell adhesion was observed and recorded in postischemic venules in the soft-tissue flaps. Images were recorded for an average of 5 venules in each flap and for 1 minute in each venule (Figure 2).

During recording, flaps were moistened by means of superfusion with bicarbonate-buffered saline solution. A solution with 0.02% rhodamine 6G was given intravenously to label leukocytes such that they could be observed by means of fluorescence using intravital microscopy. Similarly, donor platelets were labeled with carboxyfluorescein succinimidyl ester and administered intravenously; the platelets could be observed by means of fluorescence using intravital microscopy. Rhodamine- and carboxyfluorescein succinimidyl ester–labeled platelets (measured as $10^6$ per microliter) were administered via the internal jugular vein 10 minutes before recording of venules by intravital microscopy. The variables measured were rolling and adherence of leukocytes and saltation and adherence of platelets in a 1-minute period in each venule. Leukocyte and platelet activities were compared for various ischemia and reperfusion times. Statistical data analysis was in the form of paired, 2-tailed $t$ tests, and $P < .05$ was considered significant.

**RESULTS**

The rate of rolling leukocytes was not significantly different for the 15-, 30-, or 60-minute ischemia groups after 30 minutes of reperfusion compared with the sham ischemia group. In the 90-minute ischemia group, the rate of rolling leukocytes was significantly greater than that of the sham ischemia group (Table). The rate of leukocyte adherence to venule walls was significantly greater for the 15-, 30-, and 60-minute ischemia groups after 30 minutes of reperfusion compared with the sham ischemia group (Table). No significant difference was found in the rate of platelet saltation or adherence across all ischemia groups after 30 minutes of reperfusion compared with the sham ischemia group (Table). Similarly, after 4 hours of reperfusion, no significant difference was found in the rate of leukocyte rolling or adherence or platelet saltation or adherence during all ischemic periods, with the exception of a decreased rate of platelet saltation after 90 minutes of ischemia compared with the sham ischemia group (Table).

The average rate of leukocyte rolling after 90 minutes of ischemia was significantly greater after 30 minutes of reperfusion compared with 4 hours (Figure 3).

<table>
<thead>
<tr>
<th>Ischemia Time, min</th>
<th>Leukocyte Rolling After 30 min of Reperfusion</th>
<th>Leukocyte Adherence After 30 min of Reperfusion</th>
<th>Platelet Saltation After 30 min of Reperfusion</th>
<th>Platelet Adherence After 30 min of Reperfusion</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>0 (Control)</td>
<td>7.52</td>
<td>1.10</td>
<td>2.99</td>
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<td>15</td>
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<td>2.41$^a$</td>
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<td>1.71</td>
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<tr>
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<td>2.32$^a$</td>
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<td>10.26</td>
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<td>12.33</td>
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<table>
<thead>
<tr>
<th>Ischemia Time, min</th>
<th>Leukocyte Rolling After 4 h of Reperfusion</th>
<th>Leukocyte Adherence After 4 h of Reperfusion</th>
<th>Platelet Saltation After 4 h of Reperfusion</th>
<th>Platelet Adherence After 4 h of Reperfusion</th>
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<td>2.87$^a$</td>
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</table>

$^a P < .05$ compared with controls.
The average rate of leukocyte adherence after 0, 15, and 30 minutes of ischemia was significantly greater after 4 hours of reperfusion compared with 30 minutes (Figure 4). The average rate of platelet saltation after 0 and 15 minutes of ischemia was significantly greater after 4 hours of reperfusion compared with 30 minutes (Figure 5). The average rate of platelet adherence after 0, 15, 30, and 60 minutes of ischemia was significantly greater after 4 hours of reperfusion compared with 30 minutes (Figure 6).

**COMMENT**

Transient deficient blood flow followed by reestablishment of blood flow can harm tissues. This phenomenon is known as ischemia-reperfusion injury, which is quite frequently the terminal and irreversible event resulting in tissue death in head and neck reconstructive surgery. This result is particularly true for microvascular free tissue transfer. The changes seen within the microvasculature as a result of ischemia-reperfusion injury are similar to those within inflamed tissues. Inflammatory mediators accumulate, neutrophils and platelets are recruited and activated, vascular permeability increases, and oxygen free radicals and proteolytic enzymes are released. The end result can be cellular necrosis in posts ischemic tissues. Increased platelet and leukocyte activity has been implicated in ischemia-reperfusion injury in various tissues, such as the liver, heart, and gastrointestinal tract. Various pharmacological interventions to decrease the inflammatory response of ischemia-reperfusion injury have been studied in these tissues. We present an animal model to investigate cellular dynamics within the microvasculature that occur after various periods of ischemia and reperfusion in cutaneous flaps. This study demonstrates that, after a 4-hour period of reperfusion of ischemic cutaneous flaps, platelet and leukocyte adherence to the microvasculature increased in contrast to the adherence pattern seen after a shorter reperfusion period. Leukocyte rolling activity appeared to occur at a greater rate after a longer period of ischemia and a shorter period of reperfusion, whereas a greater rate of platelet saltation oc-
curred after a shorter period of ischemia and a longer period of reperfusion.

The characteristics of platelet and leukocyte activity in the microvasculature of cutaneous flaps defined in this animal model may be used when investigating the effectiveness of interventions that have the potential to decrease the deleterious effects of ischemia-reperfusion injury. This model could also be used to investigate further the cellular and molecular events and mechanism of injury in cutaneous flaps subjected to ischemia-reperfusion injury.

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REFERENCES