Granulocytes and No-Reflow Phenomenon in Irreversible Hemorrhagic Shock

Jorge Barroso-Aranda, Geert W. Schmid-Schönbein, Benjamin W. Zweifach, and Robert L. Engler

Recent evidence shows that circulating granulocytes play an important role in capillary stasis and tissue injury. We investigated two aspects of the problem in a Wiggers hemorrhagic shock model of the rat: the survival rate and the microvascular no-reflow phenomenon. A conventional group of rats with normal blood cells and a neutropenic group of rats pretreated with intraperitoneal antigranulocyte antibody were used to evaluate the effects of granulocytes. Two hemorrhagic shock protocols (HSP) were carried out. In HSP-1, the rats were subjected to 40 mm Hg mean arterial pressure for 3 hours. The conventional group (n = 11) showed a 36% survival rate compared with 100% in the neutropenic group (n = 6). In HSP-2, the hypotension was more severe, 30 mm Hg mean arterial pressure for 7 hours. There were no survivors in the conventional group (n = 8), compared with a 100% survival rate in the neutropenic group (n = 6). The extent, location, and mechanism of the no-reflow phenomenon was investigated by examining histological sections from several organs after infusion of a contrast medium to mark vessels with flow in a control group without shock and in the HSP-2 model 2 hours after blood replacement. The arterioles and venules uniformly contained contrast medium in all three groups; only capillaries showed no-reflow. A significantly higher percentage of no-reflow was observed in the capillaries of the conventional shock group than in the neutropenic shock group. We concluded that the obstruction of capillaries was largely due to trapped granulocytes, suggesting that these leukocytes play a key role in the capillary no-reflow phenomenon and survival from hemorrhagic shock. (Circulation Research 1988;63:437-447)

Although hypovolemic shock is a multifaceted problem characterized by various forms of pathology, a key feature is the progressive deterioration of microcirculatory homeostasis; however, no consensus exists regarding the mechanisms involved. We investigated a hypothesis that involves the contribution of granulocytes. Because of their large size, circulating granulocytes require considerable deformation during their passage through capillaries, and they have a natural tendency to adhere to the vascular endothelium. Recent evidence concerning the dynamics of granulocytes in small blood vessels indicates that these cells may be intimately involved in the ischemic injury associated with hemorrhagic shock. Granulocytes protect the body from infectious diseases, but they are also known to elaborate and to contain a number of highly toxic substances, such as oxygen free radicals, N-chloramines, and lytic enzymes so that when trapped and activated in the microcirculation they may actually injure tissues. Such a situation could prevail when the capillary perfusion pressure is reduced by blood loss or trauma and when local ischemia is associated with atherosclerotic obstructions. Granulocytes have also been shown to be a key factor in the incomplete restoration of blood flow after a period of ischemia ("no-reflow" phenomenon).

The purpose of this study was to investigate the extent to which granulocytes in the microcirculation contribute to the irreversible trend of hemorrhagic shock, especially their influence on the no-reflow phenomena in several tissues. For this purpose, a comparison was made of the response of a conventional, untreated group with a neutropenic group to a Wiggers type of hemorrhagic shock. Several specific questions were addressed: 1) Does neutropenia affect survival after blood replacement measures? 2) What is the degree of no-reflow and capillary obstruction in conventional and neutropenic...
nic animals that are subjected to hemorrhagic shock? 3) Do these phenomena develop in particular organs and are they related to the presence of granulocytes?

**Materials and Methods**

**Animal Preparation**

Male Wistar rats ranging in weight from 200 to 300 g were subjected to a modified Wiggers hemorrhagic shock protocol. Two different groups of rats were studied, a conventional group with normal blood cells and a neutropenic group. Rats were maintained on a conventional pellet diet before the experiment and were anesthetized with pentobarbital (45–50 mg/kg i.m.). Catheters were placed in the femoral artery and vein with PE-50 polyethylene tubing. To minimize coagulation, heparin was administered intravenously 30 minutes before the experiment in a dose of 100 units/ml estimated blood volume. This dose of anticoagulant was used to prevent coagulation in the collected blood that was then reinfused after several hours, especially in the 7-hour hypotension experiments.

**Neutropenia**

Three methods were tested to induce neutropenia: 1) a miniature version of a human leukocyte filter, 2) hydroxyurea, and 3) a sheep antigranulocyte antibody specific for Wistar rats.

The effect of leukocyte filter depletion was tested in vitro. With a perfusion pressure of 100 mm Hg and a cotton density of 0.4 g/cm³, a 79% depletion of leukocytes is obtained; granulocyte depletion is 93%, and mononuclear cell depletion is 65%. However, it was not possible to bring about an in vivo state of neutropenia with these filters. The rat is an animal species with extraordinarily large storage pools of granulocytes that are released during the filtration procedure. As a result, it was not possible to reduce the circulating granulocyte counts below 1,000 cells/ml of blood over a filtration period of several hours in a group of six animals.

In another set of experiments (n = 4), rats received an intravenous (jugular vein) injection of 220 mg/kg hydroxyurea for several days until the peripheral blood granulocyte count was decreased to less than 10% of the baseline value or to an absolute count of less than 250 cells/mm³. Hydroxyurea was administered also on the day of the study. This procedure requires 4–6 days. Total leukocyte counts fell by 72%, while the granulocyte count decreased by 90–95%, and mononuclear cells decreased by 64–67%. The neutropenic state, however, is maintained only for about 100 minutes after cannulation and is then followed by a conspicuous rise of cell counts. This method was not considered to be appropriate for this study.

We then decided to use sheep anti-Wistar rat polymorphonuclear-immunoglobulin (Pharmacia AB, Uppsala, Sweden). The antigranulocyte antibodies were injected intraperitoneally at a dose of 3–4 ml/kg about 4 hours before the experiment. This route of administration avoids the intravenous injection of the antibody, which generally leads to pulmonary granulocyte accumulation and respiratory complications. A set of three normal rats were followed for 6 hours to obtain details of leukocyte and differential counts. The granulocyte count was found to remain consistently below 200 cells/mm³. Total leukocyte counts fell by 30–90%, while granulocyte counts decreased between 92 and 98%, and mononuclear cells were decreased between 30 and 90% with no apparent effect on platelets or red blood cells.

**FIGURE 1.** Photomicrograph of myocardium cross section of a rat in the control or no-shock group. All capillaries show reflow (contain carbon). Two arterioles show reflow. Occasionally, capillaries with a few carbon particles show reflow. In black-and-white photographs, it is not possible to distinguish carbon particles from other darkly stained cell structures, but carbon particles are readily recognized by their brown color in light microscopy.
cells. Inasmuch as granulocyte number was reduced for at least a 6-hour period, the procedure was considered adequate to serve as a neutropenic model in this study.

**Experimental Protocol**

After cannulation, the rats were observed for 20 minutes to allow mean arterial pressure (MAP) and heart rate to stabilize. Hypotension was induced by a stepwise reduction in the blood volume through a femoral artery catheter during a period of 40 minutes (bleeding period) to a prescribed pressure level (40 or 30 mm Hg). Thereafter, small aliquots of blood were either removed or injected to keep MAP within this specified range for a given period of time.

Two hemorrhagic shock protocols (HSP) were carried out, one at 40 mm Hg of mean arterial pressure for 3 hours (HSP-1) and the other at 30 mm Hg for 7 hours (HSP-2). Two hemorrhagic shock protocols were used for several reasons. HSP-1 was used to determine whether the 24-hour survival statistics after blood replacement are improved in neutropenic animals compared with partial mortality in the conventional group. The HSP-2 model, providing a conventional group with 100% mortality, was a more stringent test for the efficacy of neutropenia and for the extent of the no-reflow phenomenon. Approximately 50% of the antibody-treated rats (neutropenic group) and 20% of the conventional group died during the hypotensive period because of excessive gastrointestinal bleeding in both shock protocol models. In the experiments involving a 7-hour period of hypotension (HSP-2), the blood pressures of those animals that on autopsy were found to have gastrointestinal bleeding could not be maintained at the prescribed level of hypotension without repeated injections of blood to the extent that essentially the entire volume of withdrawn blood had been restored. Animals that died during the hypotensive period were consistently found at autopsy to have a substantial volume of blood in the lumen of the stomach and small intestine. These animals were excluded from the study.

The blood that was removed during the hemorrhagic procedure was stored at 4°C during the shock period in a closed syringe. At the end of the shock period, the blood was warmed in a 40°C bath and returned by slow intravenous infusion during a period of 10 minutes. The animals were then observed for 2 hours. The catheters were then sealed and placed underneath the skin incision, after which the incisions were surgically repaired, and the animals were observed for a period of 24 hours to determine survival. Surviving animals were killed 24 hours after the shock protocol by inhalation of metofane.

**Measurements**

The following indexes were recorded: MAP, heart rate, amount of blood removed and injected, central hematocrit level, leukocyte count, and differential white cell count.

All data were recorded at regular time intervals. Statistical comparison between different groups of animals was carried out on the basis of the median or means of individual sets of variables within each group (Mann-Whitney and Student’s t test).

**Carbon Reperfusion Studies**

In this experiment, the extent, location, and mechanism of the no-reflow phenomenon in irreversible hemorrhagic shock were determined in several organs after infusion of a contrast medium to mark vessels.
with flow in three groups of rats: a control group without shock, a conventional shock group, and a neutropenic shock group 2 hours after blood replacement. HSP-2 was the shock model used for this aspect of the study.

The carbon reperfusion technique was carried out in two steps:

1) Blood cells and circulating fibrinogen were washed out by infusion of Ringer's solution to prevent coagulation in the presence of the contrast medium. This initial step was carried out immediately after general anesthesia of the rat. Ringer's solution was then infused at 40°C through the femoral artery at a reservoir pressure of 200 mm Hg to obtain a pressure of about 120 mm Hg at the end of the catheter, and coincidentally, an amount equal to the infused solution was removed from the femoral vein. Washout of the blood requires between 6 and 8 minutes as evidenced by the fact that the fluid draining from the femoral vein contained only traces of red cells.

The Ringer's solution was prepared as follows: Plasma-Lyte A (electrolyte solution, pH 7.4, 294 mosm/l); bovine albumin 2.5 gm/100 ml; heparin 10 units/ml of solution; potassium chloride to increase the concentration of potassium ions in the solution to 30 meq; this concentration induced cardiac arrest within 3–5 minutes, minimizing the time required to remove the blood and resulting in no visible swelling of the tissues; sodium nitroprusside 100 μg/100 ml solution was included as a vasodilator; and finally, distilled water was used to adjust the osmolality to 300 mosm.

2) Infusion of contrast medium marked the flowing vessels. Blood washout is followed immediately without allowing a pressure loss by the infusion of a carbon suspension (Pelikan, 17 black India drawing ink, FRG, in Plasma-Lyte A, ratio 1:2) at 40°C through the femoral artery at 200 mm Hg for 15 minutes. As indicated during the infusion, an equivalent volume was withdrawn from the femoral vein.

**Tissue Histology**

After the carbon infusion, the abdominal cavity and the chest were opened and the carcass was immersed in AFC solution (87% ethanol, 10% formaldehyde, and 3% glacial acetic acid) and fixed overnight. Selected tissues (heart, kidney, pancreas, abdominal muscle, and triceps brachii) were removed and cut into 1.5-mm-wide slices, fixed for an additional 3 hours in AFC solution, and then washed with cacodylate buffer (pH 7.4, 350 mosm). Biopsy specimens (8–15) from different regions in each tissue were trimmed to about 1.5 × 1.5 × 4 mm, postfixed in 1% OsO₄, dehydrated in graded ethanol, and embedded in araldite resin.

In the case of heart and skeletal muscle, the tissues were oriented so that the fiber direction was parallel to the length of the tissue block and so that specimens would yield predominantly cross sections. The tissues in kidney and pancreas were not oriented because all sections contain large numbers of cross sections. Sections of 1-μm thickness were made and stained with 1% toluidine blue and viewed through a light microscope with a high-resolution oil-immersion objective (×63, 1.4 n.a.) and ×10 eyepiece, which permits recognition of capillaries, endothelial cells, carbon particles, platelets, and red and white blood cells.

Histological inspection was used to determine the fraction of arterioles, capillaries, and venules in which reflow was present (i.e., vessels with carbon) and to determine the average frequency of leukocytes in capillaries.13
Wherever carbon was observed in a blood vessel, the entire vessel was considered perfused. When no carbon was observed in the vessel, the blood vessel was considered nonperfused (Figures 1–3). The total number of capillaries, the number of capillaries without carbon, and the number of capillaries containing a granulocyte were recorded. A nonperfused capillary was identified unequivocally only when the endothelial cell was recognizable as surrounding an open lumen. The criteria for identification of a granulocyte were that the granulocyte nucleus and cytoplasm had to be recognizable together with the surrounding endothelial cell (Figure 2).

The extensive histological procedure was performed on a set of three rats for each group. Although only three rats were used for every group, the data for each animal were obtained from approximately 18,000 capillaries.

Results

Survival Statistics

The 24-hour survival rate after blood replacement for HSP-1 was 36% for the conventional group \((n = 11)\) and 100% for the neutropenic group \((n = 6)\). Survival during the recovery period for HSP-2 was 0% in the conventional group \((n = 8)\) and 100% in the neutropenic group \((n = 6)\).

Blood Pressure and Heart Rate

MAP during the control period in HSP-1 averaged 117±7.0 mm Hg and in HSP-2 averaged 106±6.5 mm Hg. Initially, MAP was restored to near-control levels by the blood replacement in both groups (conventional and neutropenic) subjected to HSP-1 and HSP-2 regimens. Shortly thereafter, two different trends were observed. Systemic pressure fell gradually throughout the 2-hour postshock period in the conventional group subjected to both hemorrhagic shock protocols. At the end of 2 hours postreplacement, the conventional group showed a 14% lower MAP in HSP-1 and a 31% lower MAP in HSP-2 than in the neutropenic group (Figures 4 and 5). The differences in MAP for HSP-2 between the conventional and neutropenic groups were statistically significant (Figure 5; \(p < 0.05\) by Mann-Whitney test).

In the HSP-1 conventional group of 11 rats, four survived and seven animals died. At the end of 2 hours after replacement, MAP was 15% lower in the nonsurvival animals than in the survival animals. No significant differences could be detected in the heart rate response between the conventional and neutropenic groups in both of the shock protocols. Heart rate was reduced during the bleeding procedure and during the hypotensive shock period in both shock protocols (Figures 4 and 5). Heart rate was lower in neutropenic rats than in conventional rats during the hypotensive period, although the trend is not statistically significant for the number of animals studied. With blood replacement, a tachycardia developed in HSP-1. In HSP-2, the heart rate increased slightly in both groups but remained still lower than the mean control value.

Bleed-Out Volume to Achieve Hypotension and Its Maintenance

There were no significant differences in the amount of blood that had to be withdrawn to bring MAP into the 30 or 40 mm Hg range for the conventional and neutropenic groups.

No significant differences could be detected in the maximum blood volume withdrawn during the procedure between the two groups in HSP-1, although the conventional group tended to have a higher value than the neutropenic group (Figure 4). In HSP-2, a maximum blood volume withdrawn of 3.80±0.20 ml/100 g body wt was reached in the conventional group at 102±20 minutes after the initiation of bleeding (Figure 5). For the neutropenic group, no significant differences could be detected in mean arterial pressure (MAP) after 2 hours of blood replacement between the conventional and neutropenic group. HCT, hematocrit; HRT RATE, heart rate; \(\Delta V\), blood removed.
FIGURE 5. Graph of conventional group (n=8) with 100% mortality, neutropenic group (n=6) with 100% survival. Hemorrhagic shock protocol-2 consisted of four periods: I, control period (20 minutes); II, bleeding period (40 minutes); III, hypotensive period (7 hours at 30 mm Hg); and IV, postshock observation period (2 hours). The pressure after 2 hours of reperfusion was higher (*p<0.05) and the volume to maintain pressure was lower (**p<0.01) in neutropenic animals. HCT, hematocrit; AV, blood removed; HRT RATE, heart rate; and MAP, mean arterial pressure.

**Blood Counts**

Hematologic variables are shown in Figures 4–7. The hematocrit level in the conventional group in HSP-2 at 2 hours after blood replacement was 17% higher than the mean control value (p<0.05).

The granulocyte count during the control period was 1.5 ± 0.4 x 10^3 cells/mm^3 in the conventional group and 0.03 ± 0.02 x 10^3 cells/mm^3 in the neutropenic group for HSP-1 (p<0.005). In HSP-2, the granulocyte count during the control period was 1.5 ± 0.6 x 10^3 cells/mm^3 in the conventional group compared with 0.1 ± 0.07 x 10^3 cells/mm^3 in the neutropenic group (p<0.005). The granulocyte count for the neutropenic group remained below 300 cells/mm^3 throughout the experiments for both hemorrhagic shock protocols (Figures 6 and 7).

No significant differences could be detected in the granulocyte count between survivals and nonsurvivals for the conventional group in HSP-1.

**Histology**

Histological analysis showed reflow in all arterioles and venules of the various organs under observation in the three groups; control or no-shock, conventional shock, and neutropenic shock (Figures 1–3). Evidence of no-reflow was observed only in the capillaries (Figure 8). A higher percentage of capillaries developed no-reflow in the conventional shock group in heart, kidney, skeletal muscle, and pancreas than in the neutropenic shock group (p<0.005). These differences in capillary reperfusion are illustrated in the case of the heart in Figures 1–3. The histological sections showed a random mixture of capillaries with and without carbon (Figure 2). No extravasation of carbon was observed (Figures 1–3). No platelet plugs or aggregates of erythrocytes could be detected in no-reflow capillaries. The number of sections used and the number of capillaries that were counted are shown in Table 1. Although sections of the brain were examined, no reliable histological analysis could be carried out on the plastic embedded sections because of the difficulty of recognizing endothelial cells in vessels without carbon and of distinguishing them from the parenchymal tissue.

The frequency with which leukocytes were trapped in the capillaries for the organs studied (Figure 9) is significantly higher in the conventional shock group than in the neutropenic shock group (p<0.005) and also significantly higher than in the control nonhemorrhagic group (p<0.005).

The average deformed length of the trapped granulocyte in the capillaries of heart skeletal muscle, pancreas, and kidney was 10.0 ± 1.2 μm (n = 10), 12.3 ± 2.0 μm (n = 10), 10.1 ± 1.6 μm (n = 10), and 9.0 ± 1.6 μm (n = 10), respectively.

The average distance between leukocytes along a capillary (the majority were granulocytes) in the group a maximum blood volume withdrawn of 3.39 ± 0.24 ml/100 g body wt was reached at 90 ± 20 minutes after bleeding initiation (Figure 5). This difference between the two groups was statistically significant (p<0.05). The slight difference in the two shock protocol groups may be due to seasonal variations known to affect the shock response. HSP-1 was studied in the summer, and HSP-2 was studied in the winter months.

On average 44.4 ± 10.3% of the maximum shed volume had to be reinjected in small aliquots to maintain the pressure at 40 mm Hg for the nonsurviving animals, as contrasted with 22.8 ± 4.7% for survival animals.
no-reflow capillaries is defined as the number of capillaries without carbon divided by the number of capillaries with leukocytes and multiplied by the average granulocyte deformed length in the capillaries for each organ (Table 1). For the conventional shock group, the mean interleukocyte distance (100–152 μm/white blood cells) was significantly less than in the neutropenic shock group (229–399 μm/white blood cells) \((p<0.02)\), and also was significantly less than in the control group (202–331 μm/white blood cells mean) \((p<0.05)\). No significant differences could be detected in this regard between controls and the neutropenic shock group. The average distance between leukocytes in the no-reflow capillaries served as a measure for the accumulation of granulocytes in the capillaries. Overall counts of granulocytes are lower in the controls and neutropenic shock rats than the conventional shock rats.

The correlation between the fraction of total capillaries without carbon (no-reflow) and the fraction of total capillaries with leukocytes was 0.82 \((p<0.001, n=54 \text{ mean values from all studied tissues})\) (Figure 10).

**Discussion**

**Granulocytes in the Microcirculation During Hemorrhagic Shock**

Several new findings have been documented in this study. 1) The substantial improvement in survival after blood replacement in neutropenic animals is unequivocal evidence that granulocytes play an important role in the progressive inability to recover from hemorrhagic shock. 2) A capillary no-reflow phenomenon was demonstrated in a wide range of tissues after hemorrhagic shock in conventional animals. 3) The fact that the no-reflow phenomenon is practically eliminated in most tissues during granulocyte depletion (neutropenic group) strengthens the hypothesis that leukocyte capillary plugging is the major factor.

The clear-cut differences in the survival after hemorrhagic shock between the conventional and neutropenic group appears to be related to the extent to which the no-reflow phenomenon develops. It has been presumed that granulocytes actively contribute to ischemic damage by either biochemical activation (formation of oxygen free radicals) within the microcirculation, and by mechanical plugging of the microcirculatory flow. Additional evidence indicates that granulocytes increase capillary...
permeability during acute ischemia with loss of the circulating blood volume.15,16

It should be emphasized that all arterioles and venules could be reperfused in all tissues of the three groups and only vessels with diameters below about 10 μm, that is, the capillaries, developed no-reflow. This observation confirms the thesis that the impairment of local tissue perfusion in hemorrhagic shock resides in the narrow capillaries as has been observed by other investigators.17,18 The distribution of nonperfused capillaries was seemingly random with perfused and nonperfused capillaries often side by side. Comparable observations have been made in ischemic heart.13,15 In none of the no-reflow capillaries was there evidence of aggregates of erythrocytes or swelling or elastic recoil of capillary endothelium to the point of lumen closure. Platelet plugs were, likewise, not seen in capillaries, which is consistent with the findings in the acutely ischemic heart.13

Granulocyte entrapment has been reported frequently to occur at a tapering segment of the capillary network, such as the entrance to a capillary,18 or at a point along the capillary where the endothelial nucleus protrudes.7 In the present study, leukocyte entrapment occurred much more frequently in the capillaries of the conventional shock group than in the neutropenic shock group. There is a direct correlation between the fraction of capillaries without carbon (no-reflow) and the average leukocyte frequency counts. These findings, together with the fact that the percentage of capillaries with no-reflow in the conventional shock group was significantly higher than in the neutropenic shock group, further reinforce the concept that leukocyte capillary plugging is a key factor in the no-reflow phenomenon in hemorrhagic shock.

If a single leukocyte lodged in a capillary in the ischemic area was sufficient to block flow totally, then only one leukocyte per unbranched capillary would be expected. The average capillary length per leukocyte in which there was no reflow for the tissues studied in the conventional shock group ranged from 100 to 155 μm/leukocyte (mean values). These values are the same order of magnitude as for the values of the unbranched capillary length of about 100 μm in the epicardium.19 The present findings thus support the conclusion that there is about one leukocyte for each "no-reflow" capillary as observed in skeletal muscle during shock2 and in ischemic heart.13 Restoration of arterial pressure to normal values by itself is apparently not sufficient to remove such trapped granulocytes. This feature may be the result of enhanced perivascular edema and the increase in granulocyte adhesion to the endothelial wall. In view of the strict criteria used for identification of leukocytes, it is likely that we have underestimated the actual frequency of leukocytes in the light-microscopy analysis of our biopsy specimens.

**Neutropenia Procedure**

Several possible procedures have been used in the rat to induce neutropenia, such as administration of 100 to 155 μm/leukocyte (mean values).

**FIGURE 8. Histogram of percent capillaries with no-reflow (without carbon) from heart muscle (left and right ventricle), kidney, pancreas, triceps brachii, and abdominal muscle in the three groups: control or no-shock (n=3), conventional shock (n=3), and neutropenic shock (n=3). Number of biopsies used and the number of capillaries that were counted are shown in Table 1. *p<0.005 (Mann-Whitney) conventional shock vs. neutropenic shock; **p<0.005 conventional shock vs. control; tp<0.01 neutropenic shock vs. control.

**TABLE 1. Average No-Reflow Capillary Length Per Leukocyte**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control no-shock (n=3)</th>
<th>Conventional shock (n=3)</th>
<th>Neutropenic shock (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricle</td>
<td>331 ± 168</td>
<td>121 ± 41</td>
<td>399 ± 166</td>
</tr>
<tr>
<td>(22,913;12)</td>
<td>(24,369;18)</td>
<td>(24,973;12)</td>
<td></td>
</tr>
<tr>
<td>Right ventricle</td>
<td>321 ± 95</td>
<td>137 ± 41</td>
<td>387 ± 145</td>
</tr>
<tr>
<td>(12,774;6)</td>
<td>(11,900;6)</td>
<td>(13,986;8)</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>202 ± 74</td>
<td>111 ± 72</td>
<td>229 ± 91</td>
</tr>
<tr>
<td>(5,354;12)</td>
<td>(3,796;13)</td>
<td>(4,080;12)</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>276 ± 95</td>
<td>152 ± 82</td>
<td>335 ± 195</td>
</tr>
<tr>
<td>(6,732;13)</td>
<td>(5,570;11)</td>
<td>(5,155;10)</td>
<td></td>
</tr>
<tr>
<td>Abdominal muscle</td>
<td>245 ± 87</td>
<td>100 ± 53</td>
<td>307 ± 180</td>
</tr>
<tr>
<td>(2,813;11)</td>
<td>(2,383;13)</td>
<td>(3,433;12)</td>
<td></td>
</tr>
<tr>
<td>Triceps brachii</td>
<td>261 ± 78</td>
<td>145 ± 61</td>
<td>317 ± 97</td>
</tr>
<tr>
<td>(3,029;7)</td>
<td>(3,879;12)</td>
<td>(5,583;10)</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD in micrometers. n = number of animals.

Figures in parentheses indicate the number of capillaries that were counted and the number of sections used. No-reflow capillaries are vessels without carbon.

*p<0.004 (Mann-Whitney) conventional vs. control; tp<0.002 conventional vs. neutropenic; tp<0.05 conventional vs. control; fp<0.02 conventional vs. neutropenic.
tion of cytotoxins, whole body radiation, or antigranulocyte immunoglobulins, among others. None of these methods provides an ideal way to deplete circulating granulocytes. Each procedure is associated with some deterioration of the general state of health, and in some cases, this deterioration is severe.

In the present investigation when the primary need was for an acute decrease in circulating granulocytes, the use of antigranulocyte antibodies was found to be the preferred method in spite of a number of limitations. 1) Only limited control is possible concerning where the circulating antibody-labelled granulocytes will be sequestered, and there is a risk that the complexed cells may become activated. 2) Administration of the antigranulocyte antibodies may produce effects other than a neutropenia. Varying numbers of mononuclear cells are also depleted. 3) When antigranulocyte antibodies are given intraperitoneally, a local inflammatory reaction is produced to the extent that almost half of the antibody-treated animals develop intestinal bleeding during hemorrhagic shock. This effect appears to be due to the entrapment of the slowly diffusing granulocyte-antibody complex around the small intestine; possibly related to the serum. In this study, we did not take the precaution to empty the gastrointestinal tract of food before antibody treatment. It is possible that the deaths due to intestinal congestion and bleeding could have been reduced by such a procedure as in experiments reported by Smith and coworkers.9 4) A small percentage (5%) of the rats treated with antigranulocyte antibodies showed a deterioration of their general state of health accompanied by a below normal hemodynamic status. Evidently, circulating "neutropenia" is achieved only with some compromise.

Animals with a depleted circulating granulocyte count can withstand long periods of ischemia; in the present series of experiments, these animals survived after 7 hours at 30 mm Hg, but they might have survived for an even longer period if the test had been continued. After recovery, the neutropenic animals were alert, drinking, eating, walking, and climbing.

Although the use of the antigranulocyte antibody to decrease the circulating cell count serves to clarify the mechanisms involved in shock, it does not necessarily imply that the procedure is a therapeutic intervention.

Heart Rate

During the course of the experiments in both conventional and neutropenic cohorts, a decrease in heart rate was recorded. Bradycardia during hemorrhagic shock has been reported in humans,20,21 in cats,22 and in rats.23 Öberg and Thorén22 and Secher et al20 suggest a vagal withdrawal mechanism to explain the bradycardia.

Reinfusion During Shock

A much smaller percentage of the maximum shed volume per body mass was reinjected during shock to maintain the pressure at 30 mm Hg in the
neutropenic group than in the conventional group in HSP-2. It was 48% lower. It is likely that either an extravascular loss of fluid develops in the conventional group during hypotension or that trapping of blood on the venular side occurs so that a greater volume replacement is needed to maintain pressure at the prescribed hypotensive level than in neutropenic animals.

Engler et al.\(^1\) have suggested that trapped leukocytes have a direct effect on transcapillary filtration during acute ischemia. They found an increase in the tissue water content in the myocardium during ischemia in dogs with normal leukocyte levels, but no such edema in leukocyte-depleted animals. A linear correlation existed between water content and granulocyte content in individual biopsies.\(^2\) Hernandez et al.\(^3\) have reported that both neutrophil depletion and prevention of neutrophil adherence significantly attenuate the increased microvascular permeability induced by ischemia-reperfusion in the bowel. A whole array of inflammatory products have been identified that can lead to elevated tissue permeability in the presence of granulocytes.\(^4\)–\(^7\)

Capillary and Postcapillary Granulocytes

A substantial number of granulocytes can be observed by intravital microscopy residing in a marginal zone adjacent to the postcapillary and venular endothelium. This typical situation is also observed under low-flow conditions and inflammatory reactions. The granulocytes that are trapped in the capillary network during hypotension probably constitute only a small fraction of the total population. Thus, although the capillary cell pool may be the major contributor to the no-reflow phenomena, the venular pool may also contribute to the elevated whole-organ resistance and serve to exacerbate the biochemically mediated injury to the tissue parenchyma. One of the consistent observations in hemorrhagic shock is the early disappearance of the granulocytes from the circulating pool, the so-called "flight of the granulocytes".\(^8\) The current theory is consistent with this observation. Once the granulocytes are trapped in capillaries, drugs and agents used to dislodge them are less effective because of inaccessibility of local capillary regions because of the obstruction. The dislodgement of the granulocytes is further hampered by adhesion to the endothelium.

Still unresolved are details of the regional distribution of the granulocytes that leave the circulating pool during the initial stages of the ischemic state. Such information is essential for an understanding of the mechanism of granulocyte activation in hemorrhagic shock. Identification of the precise mechanism by which granulocytes lower the survival after hemorrhagic shock should lead to improved therapy.

Acknowledgment

The polyclonal antibody against rat neutrophils was generously donated by Dr. Karl Arfors, Pharmacia, La Jolla, California.

References


Downloaded from http://circres.ahajournals.org/ by guest on April 7, 2017


27. Smedegård G: Mediators of vascular permeability in inflammation. Prog Appl Microcirc 1985;7:96–112


KEY WORDS • granulocytes • no-reflow phenomenon • neutropenia • hemorrhagic shock • capillaries
Granulocytes and no-reflow phenomenon in irreversible hemorrhagic shock.
J Barroso-Aranda, G W Schmid-Schönbein, B W Zweifach and R L Engler

doi: 10.1161/01.RES.63.2.437

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/63/2/437

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/