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Quantitative Analysis of Microcirculatory Disorders After Prolonged Ischemia in Skeletal Muscle

Therapeutic Effects of Prophylactic Isovolemic Hemodilution

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Summary. Reperfusion injury following prolonged ischemia is thought to be caused primarily by microvascular failure. The aim of the present study was to investigate whether prophylactic isovolemic hemodilution with Dextran 60 (hct 30%) could improve microvascular perfusion after 4 h of pressure-induced ischemia in skeletal muscle.

In 28 Syrian golden hamsters (6–8 weeks/60–80 g b. wt.) a dorsal skinfold chamber and permanent arterial and venous catheters were implanted under Nembutal anesthesia (50 mg/kg b. wt.). Following a recovery period of 48 h pressure-induced ischemia was applied to the skeletal muscle within the skinfold chamber by means of a transparent stamp. Quantitative analyses of microhemodynamics were performed in the awake animal prior to and 15 min, 1, 2, 4 and 24 h after ischemia using vital fluorescence microscopy.

In non-treated animals, functional capillary density decreased after 4 h of ischemia to 30% of the initial values ($P < 0.001$); after 24-h reperfusion only 50% of the initially perfused capillaries were reperfused ($P < 0.001$). The heterogeneity of functional capillary density increased after ischemia to a maximum of 2.19 ± 0.94 as compared to 0.48 ± 0.11 prior to ischemia. Capillary RBC-velocity suffered a marked reduction in the early reperfusion phase and did not recover up to the 24-h observation time. In contrast, prophylactic isovolemic hemodilution was associated with only a small and reversible reduction of functional capillary density after 4-h ischemia. At 24-h reperfusion 90% of the initially perfused capillaries were reperfused. Capillary

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RBC-velocity was reduced in the early reperfusion phase, but returned to normal values within 24 h. Thus, prophylactic isovolemic hemodilution resulted in a marked reduction of microvascular reperfusion failure in skeletal muscle. A hematocrit lower than normal prior to ischemia provides better conditions for capillary reperfusion after prolonged ischemia.

Key words: Microcirculation – Skeletal muscle – Ischemia – Reperfusion injury – Hemodilution

Introduction

The mechanisms of reperfusion injury after prolonged ischemia have not yet been revealed. In 1948, Harman [22] was the first to describe alterations of reperfusion in the microvasculature of skeletal muscle following prolonged ischemia. Similar findings were reported for the kidney by Sheehan and Davis [51]. Later on, Ames et al. [1] demonstrated that postischemic microvascular changes in the rabbit brain lead to a "point of no return"; these authors coined the term "no-reflow phenomenon". Subsequently postischemic microvascular failure was observed in the adrenals [29], kidneys [15, 55], striated muscle of rats [53], and in the myocardium of cat [30] and dog [27].

Many factors have been considered as causes of the "no reflow phenomenon", such as leukocyte-endothelium interaction [5, 12], endothelial cell swelling [18, 45], impaired blood fluidity [31, 50], and more recently oxygen-derived free radicals [19, 35, 44].

Studies from our laboratory [48] have revealed that 4-h ischemia in hamster skeletal muscle results in irreversible microvascular reperfusion injury. Comparable findings in the cat have been reported by Eriksson et al. [13] and Dunant and Edwards [9]. Santavirta et al. [49] observed an increased number of small vesicles in the cytoplasm of capillary endothelial cells occurring after 3-h ischemia in skeletal muscle. These first signs of impaired integrity of the capillaries become prominent and irreversible after 4-h of ischemia and are accompanied by hemolysis of intraluminal erythrocytes and ruptured endothelial cells [18, 49].

Normovolemic hemodilution has been suggested as a modality to reverse microcirculatory disorders by improving flow properties and the flow conditions of the blood [37, 40, 50]. A decrease in hematocrit lowers the viscosity of whole blood, thus reducing the viscous resistance to flow and enhancing venous return, which in turn increases stroke volume, while heart rate remains constant. Despite the dilutional fall of the red cell concentration and thus oxygen capacity, the enhanced cardiac output allows for a significant increase of the systemic oxygen transport capacity, which reaches a maximum at a hematocrit value of approximately 30% [37]. Due to increased total and nutritional flow, oxygen transport is maintained in the individual organs, despite the reduced hematocrit [39].

The aim of this study was to investigate whether isovolemic hemodilution with Dextran 60 could improve microvascular perfusion in skeletal muscle following prolonged ischemia.

Material and Methods

Model

Our studies were carried out in the Syrian golden hamster, equipped with a dorsal skinfold chamber, allowing intravital microscopy of the microcirculation in skeletal muscle and skin in the awake animal. In 28 animals (age: 6–8 weeks, 60–80 g b.wt.) a dorsal skinfold chamber and two permanent arterial and venous catheters were implanted. The chamber and implantation procedure have been described previously by Endrich et al. [10]. Briefly: Under Nembutal anesthesia (50 mg i.p./kg b.wt.) the animals were fitted with two symmetric teflon-coated aluminium frames, positioned on the dorsal skinfold in such a fashion that they sandwich the extended double layer of the skin. One layer was completely removed in a circular area (∅ 15 mm) and the remaining layer, containing skeletal muscle and s.c. tissue was covered with a removable cover glass, incorporated in one of the aluminium frames. Two permanent catheters were passed from the dorsal to the ventral side of the neck, one placed into the carotid artery and one into the jugular vein.

Experimental Design

Following a recovery period of 48 h the animals were divided alternately into two main groups, as follows:

- Group A (control):
  4-h ischemia, followed by repeated analysis during the first 4 h after ischemia ($n = 10$);
  4-h ischemia, followed by analysis after 24 h ($n = 8$);
- Group B (hemodilution):
  isovolemic hemodilution to a systemic hematocrit of 30% prior to 4-h ischemia. Analyses were performed during the first 4 h and at 24 h after ischemia ($n = 10$).

For technical reasons, the animals analyzed during the first 4 h in the control group were not identical with those analyzed 24 h after ischemia.

In group A analyses were performed prior to and 15 min, 1 h, 2 h, 4 h, and 24 h after ischemia. In Group B an additional analysis was carried out 30 min after hemodilution.

Hemodilution

To achieve a hematocrit of 30%, normovolemic hemodilution was performed by removal of 1.4 ml blood from the carotid artery in two steps, and infusion of 1.4 ml Dextran 60 (Macrodex 6%, Schiwa, Glandorf/FRG). During the exchange procedure mean arterial blood pressure and heart rate were monitored.

Ischemia

Ischemia was induced on the lower part of the tissue within the observation window by means of a transparent disk fixed to the skinfold chamber [48]. Under transillumination an external pressure of 40–50 mm Hg was applied to the tissue by means of an adjustable screw clamp. The pressure was regulated so as to empty the vessels within the tissue. The non-compressed tissue (upper part of the observation window) served as non-ischemic control tissue.

Macrohemodynamics

Mean arterial blood pressure (MAP) and heart rate (HR) were monitored before and after the experiment and during the process of hemodilution. Systemic hematocrit was determined before, during, and after hemodilution.

Microhemodynamics

By means of intravital microscopy, video techniques, and the computer-assisted Microcirculation Analysis System (CAMAS) [57] the microvascular perfusion outside of and within the ischemic skin muscle contained in the chamber was analysed as follows:

1. Microvascular changes in two regions of interest: Each region, encompassing a surface area of 1.25 mm^2, was divided into 3 × 3 single windows for determination of functional capillary density (FCD) (total length of capillaries with blood flow per unit area of observation). FCD is given as the mean value obtained from all nine windows. In one region a network consisting of approximately 72 capillaries can be analyzed.

Three of the nine windows per region were used for measurements of the capillary RBC-velocity.

2. Microvascular changes in six sites of interest: A site of interest was defined as an area of 0.2 mm^2, containing three collecting venules (20–60 µm diameter) for determination of venular diameter and RBC-velocity.

The measurements were confined to the skin muscle without including vessels in s.c. tissue.

For measurements of capillary density, RBC-velocity and vessel diameter, and to study microvascular permeability contrast enhancement was achieved prior to ischemia through the use of TRITC-labeled Dextran 70 (TRITC Dextran M_W 70000, Research Organics Inc./USA) (5 mg in 0.1 ml NaCl i.v.) and epi-illumination or by means of a 443 nm narrowband filter and transillumination. After ischemia epi-illumination and contrast enhancement with FITC Dextran 150 (Fluorescein Isothiocyanate-Dextran, Sigma/USA) (5 mg in 0.1 ml NaCl i.v.) was used.

Changes of microvascular permeability after ischemia were quantified by planimetry of the FITC Dextran extravasates (by means of CAMAS). Dynamic data on vessel diameter was obtained by video image-shearing technique (Image Shearing Monitor Mod 907 I.P.M., INC.; San Diego, Ca., USA). RBC-velocity measurements were performed with a Photometric Analyzer and the dual slit technique (Velocity Tracker Mod 102B I.P.M., INC.; San Diego, Ca., USA).

Calculations

To assess the degree of perfusion inhomogeneites, the index of capillary heterogeneity (HI) (distribution of local capillary density) was calculated for each region (nine windows) by the equation:

$$HI = \frac{FCD_{max} - FCD_{min}}{FCD_{mean}}$$

FCD_{max}: FCD of the window with the highest functional capillary density per region;
FCD_{min}: FCD of the window with the lowest functional capillary density per region;
FCD_{mean}: mean values obtained from all nine window, within the region.

Statistcs

Statistical tests were performed only on complete experiments (no measurements missing). All parameters were tested for normal distribution and either an analysis of variances and *t*-test (normal distribution) or the U-test (Mann-Whitney) (non-normal distribution) was used to test for significant differences between the groups. A *t*-test accompanied by Bonferroni probabilities (normal distribution) or Wilcoxon test (non-normal distribution) followed by comparisons within the groups. Differences were considered significant at the $P < 0.05$ level.

Results

Effects of Ischemia in Untreated Control Animals

Macrohemodynamics. Induction and release of ischemia did not alter either the mean arterial blood pressure or the heart rate.

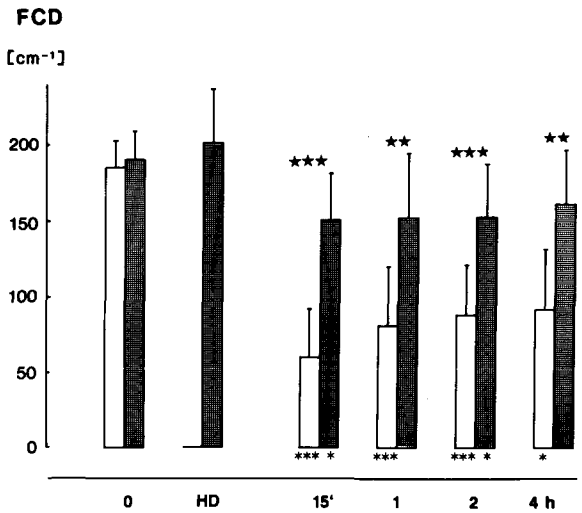

Fig. 1. Functional capillary density (FCD) prior to and at different time periods (15 min, 1 h, 2 h, 4 h) after 4 h of pressure-induced ischemia. □ Group A (control); ■ Group B (hemodilution); HD: values obtained at 30 min after hemodilution (hct 30%). Values are given as mean ± SD (each *column* represents measurements of approximately 90 windows in ten animals); Student's *t*-test (comparison between the groups); $^{**}P<0.01$, $^{***}P<0.001$. Paired *t*-test, Bonferroni correction (comparison within the group); $^{*}P<0.05$, $^{***}P<0.001$

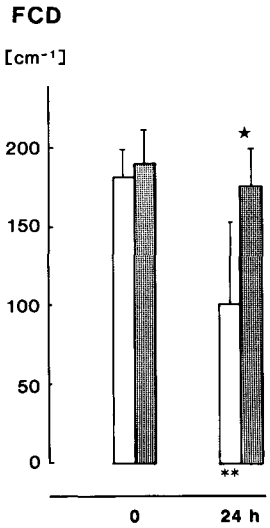

Fig. 2. Functional capillary density (FCD) prior to and at 24 h after 4 h of pressure-induced ischemia. □ Group A (control); ■ Group B (hemodilution). Values are given as mean ± SD (each *column* represents measurements of approximately 70 windows in eight animals). Student's *t*-test (comparison between the groups); $^{*}P<0.05$. Paired *t*-test (comparison within the group); $^{**}P<0.01$

Microhemodynamics. Reperfusion after 4-h ischemia was followed by a drastic decrease in FCD ($P<0.001$) (Fig. 1); during the following 24 h a slight improvement was observed. However, about 50% of all capillaries remained unperfused (Fig. 2). The heterogeneity of the local capillary density distribution increased markedly (Tables 1, 2).

Capillary RBC-velocity was reduced during the entire observation period ($P<0.001$). Fifteen minutes after reperfusion approximately 65% of the visible capillaries exhibited velocities below 0.1 mm/s (Fig. 3). At 24 h reperfusion the mean velocity was 0.19 ± 0.16 mm/s as compared to the initial value of 0.26 ± 0.09 mm/s (Fig. 4).

Table 1. Index of capillary heterogeneity (HI) as measure for the distribution of functional capillary density prior to (0, HD) and at different time periods (15 min, 1 h, 2 h, 4 h) after 4 h of pressure-induced ischemia

HI	0	HD	15 min	1 h	2 h	4 h
Control	0.48 ± 0.11		2.19 ± 0.94[d]	1.34 ± 0.74[c]	1.31 ± 0.61[c]	1.17 ± 0.87[c]
Hemodilution	0.33 ± 0.12	0.38 ± 0.14	0.60 ± 0.32[b,c]	0.80 ± 0.55	0.66 ± 0.30[a]	0.58 ± 0.18[a,c]

HD: Values obtained at 30 min after hemodilution (hct 30%)

Values are given as mean ± SD (each value represents measurements of approximately 90 windows in ten animals); Student's *t*-test (comparison between the groups); [a]$P<0.05$, [b]$P<0.01$. Paired *t*-test, Bonferroni correction (comparison within the group); [c]$P<0.05$, [d]$P<0.001$

Table 2. Index of capillary heterogeneity (HI) as measure for distribution of functional capillary density prior to and at 24 h after 4 h of pressure-induced ischemia

HI	0	24 h
Control	0.43 ± 0.11	1.37 ± 0.96[b]
Hemodilution	0.36 ± 0.05	0.48 ± 0.19[a]

Values are given as mean ± SD (each value represents measurements of approximately 70 windows in eight animals); Student's *t*-test (comparison between the groups); [a]$P<0.01$. Paired *t*-test (comparison within the group); [b]$P<0.05$

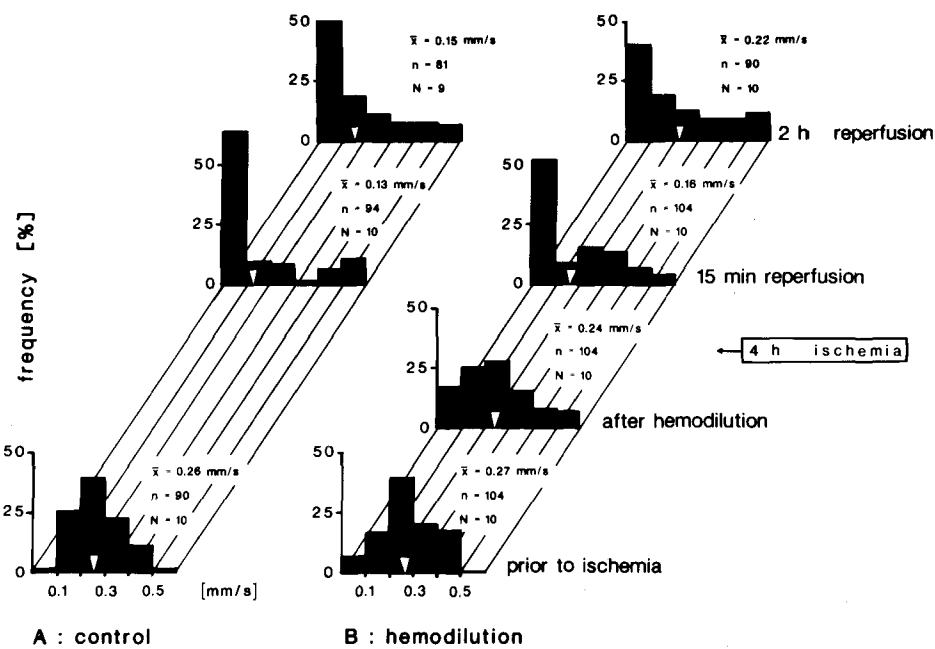

Fig. 3. Capillary RBC-velocity prior to ischemia, 30 min after hemodilution, and at 15 min and 2 h of reperfusion after 4 h of pressure-induced ischemia. In control group capillary RBC-velocity decreased significantly ($P<0.001$) after 15 min and 2 h of reperfusion; in the hemodilution group RBC-velocity was also found decreased after 15 min ($P<0.01$) and 2 h ($P<0.05$) of reperfusion (Wilcoxon-test). In the hemodilution group there was a higher capillary RBC-velocity observed after 15 min ($P<0.01$) and 2 h ($P<0.05$) of reperfusion compaired to control group (Mann-Whitney test). x = mean value; n = number of measurements; N = number of animals

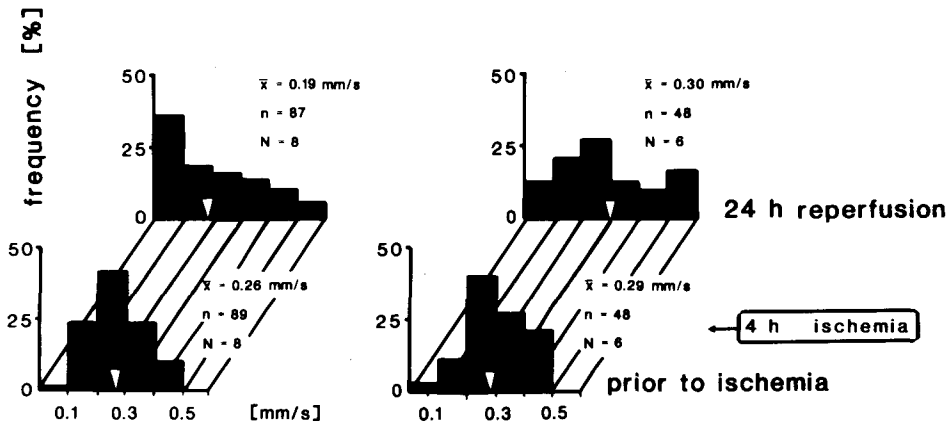

Fig. 4. Capillary RBC-velocity prior to ischemia and at 24 h of reperfusion after 4 h of pressure-induced ischemia. In control group capillary RBC-velocity was found decreased ($P<0.001$) after 24 h of reperfusion, while in the hemodilution group no changes were observed (Wilcoxon-test). In the hemodilution group a higher capillary RBC-velocity ($P<0.01$) was observed compaired to control group (Mann-Whitney test). x = mean value; n = number of measurements; N = number of animals

Table 3. Vessel diameters of collecting venules (in μm) prior to (0, HD) and at different time periods (15 min, 1 h, 2 h, 4 h) after 4 h of pressure-induced ischemia

Group	0	HD	15 min	1 h	2 h	4 h
Control ($n=39$)	30.2 (27.4/39.4)		32.9 (27.2/40.2)	33.1[a] (29.3/40.8)	35.6[b] (28.5/45.1)	35.5 (31.3/38.5)
Hemodilution ($n=38$)	35.3 (30.3/44.7)	35.2 (32.0/44.3)	37.8[c] (34.8/45.7)	39.7[c] (35.5/50.9)	38.6[c] (35.5/49.8)	41.0[c] (36.6/49.0)

HD: Values obtained at 30 min after hemodilution (hct 30%)

Wilcoxon-test (comparison within the group); values are given as median (Q1/Q3); n = number of measurements; [a] $P<0.05$, [b] $P<0.01$, [c] $P<0.001$

Table 4. Vessel diameters of collecting venules (in μm) prior to and at 24 h after 4 h of pressure-induced ischemia

Group	0	24 h
Control ($n=74$)	31.3 (27.9/39.1)	37.1[a] (30.6/44.7)
Hemodilution ($n=30$)	38.5 (30.1/47.4)	40.7 (33.7/49.8)

Wilcoxon-test (comparison within the group); values are given as median (Q1/Q3); n = number of measurements; [a] $P<0.001$

Table 5. RBC-velocity (mm/s) in collecting venules prior to (0, HD) and at different time periods (15 min, 1 h, 2 h, 4 h) after 4 h of pressure-induced ischemia

Group	0	HD	15 min	1 h	2 h	4 h
Control ($n = 39$)	0.52 (0.43/0.63)		0.28[a] (0.08/0.65)	0.36 (0.24/0.78)	0.42 (0.29/0.76)	0.57 (0.44/0.65)
Hemodilution ($n = 38$)	0.47 (0.23/1.00)	0.57 (0.18/1.35)	0.25[b] (0.06/0.86)	0.39[b] (0.05/0.81)	0.39 (0.14/0.97)	0.56 (0.08/1.08)

HD: Values obtained at 30 min after hemodilution (hct 30%)
Wilcoxon-test (comparison within the group); values are given as median (Q1/Q3); n = number of measurements; [a] $P < 0.05$, [b] $P < 0.01$

Table 6. RBC-velocity (mm/s) in collecting venules prior to and at 24 h after 4 h of pressure-induced ischemia

Group	0	24 h
Control ($n = 74$)	0.65 (0.47/0.76)	0.40[a] (0.14/0.72)
Hemodilution ($n = 30$)	0.77 (0.45/1.33)	0.67 (0.32/1.80)

Wilcoxon-test (comparison within the group); values are given as median (Q1/Q3); n = number of measurements; [a] $P < 0.001$

Though the diameters of collecting venules (20–60 μm) appeared to increase only slightly after reperfusion, this increase proved to be statistically significant, when including all the vessels measured (Tables 3, 4). The RBC-velocity in the collecting venules was reduced 15 min and 24 h after reperfusion (Tables 5, 6).

The maximum extravasation of the plasma marker (FITC Dextran 150) was observed at two hours of reperfusion, encompassing up to 14% of the ischemic tissue.

In the non-ischemic control tissue FCD was unchanged. However, there was an increase in diameters of the collecting venules ($P < 0.001$) and a reduction of flow velocity ($P < 0.05$) during the first 2 h after ischemia.

Effects of Ischemia in Diluted Animals

Macrohemodynamics. The mean arterial blood pressure (121.3 ± 5.8 mm Hg) was not altered by isovolemic hemodilution (119.2 ± 4.9 mm Hg) and no significant change was observed at the end of the experiment (119.0 ± 5.7 mm Hg). As planned, initial systemic hematocrit (45.0 ± 2.4%) was reduced by hemodilution procedure to 30.4 ± 1.8%.

Microhemodynamics. Isovolemic hemodilution with Dextran 60 resulted in a small increase in FCD. This change was not accompanied by changes of capillary RBC-velocity, but the variability of capillary blood flow values was en-

hanced (Fig. 3). The diameters and RBC-velocities of the collecting venules remained unchanged after hemodilution (Tables 3, 4).

In contrast to the control animals, reperfusion in previously diluted animals was associated with only a small and reversible reduction in FCD (Fig. 1); 24 h after ischemia 90% of the initially perfused capillaries were reperfused (Fig. 2). Capillary perfusion remained homogeneous as demonstrated by the index of capillary heterogeneity (Tables 1, 2). Capillary RBC-velocity was decreased in the early reperfusion phase (Fig. 3), but returned to normal within 24 h (Fig. 4).

As in the control animals, the diameters of the collecting venules were found increased after ischemia (Table 3). The reduction of RBC-velocity present after 15 min of reperfusion (Table 5) was fully reversed 24 h after reperfusion (Table 6).

Microvascular permeability achieved its maximum at 2 h of reperfusion, encompassing 9.5% of the ischemic area; this value was not significantly different from the changes in permeability observed in the non-diluted animals.

In the non-ischemic control tissue neither changes in FCD nor in the distribution of FCD were found. In the collecting venules RBC-velocity was significantly ($P < 0.05$) diminished during the first 2 h of reperfusion, but returned to normal within the observation period. The diameters of these vessels were increased during the entire observation period ($P < 0.001$).

Discussion

Methods for Analyzing Microcirculatory Reperfusion Failure

The no-reflow phenomenon, following prolonged ischemia, is caused by reperfusion failure in the microvasculature, as described by Ames et al. [1] in 1968. The underlying mechanisms have not yet been revealed. Difficulties in observation and quantitative analysis of the changes occuring in the microcirculation aggravate the solution of this problem.

Measurements of microcirculatory perfusion with indirect procedures, such as dyes, microspheres, Xenon radiography, or laser Doppler velocimetry, do not permit analysis of the nutritive tissue supply at the capillary level. In contrast, intravital microscopy allows assessment of nutritional capillary flow.

However, vital microscopic studies of the mesentery, tenuissimus muscle, or hamster cheek pouch suffer from the effects of anesthesia and surgical trauma; furthermore, these tissues do not permit observations for prolonged time periods.

In contrast, in the dorsal skinfold chamber of the hamster, the effects of anesthesia and surgical trauma on the microvasculature are eliminated, since a recovery period of 48 h is allowed after implantation of the chamber [11].

Acute ischemia of skeletal muscle and skin is a common clinical problem occurring as a result of tourniquet or tissue compression. To simulate the conditions of reperfusion injury following pressure-induced ischemia, a model allowing direct pressure application and observation of the microcirculation for a long time is required. In the dorsal skinfold chamber ischemia is achieved by

employing an external pressure of 40–50 mm Hg, which is sufficient to empty the blood vessels and thus renders the tissue ischemic.

Depending upon the tissue under investigation, the degree of postischemic tissue injury depends upon the time of ischemia. While in skeletal muscle 2-h ischemia leads to transient reperfusion failure, 4-h ischemia is followed by persisting microvascular reperfusion injury [48].

Reperfusion injury is characterized by impaired capillary perfusion and diminished blood flow in postcapillary venules [47]. To quantify these alterations, it is necessary to assess FCD, capillary RBC-velocity, as well as the microhemodynamics in collecting venules.

For the measurements of FCD in skeletal muscle, a region of interest (1.25 mm^2), consisting of nine single windows, with a homogeneous distribution of six to nine perfused capillaries, was selected ($\times$ 460). RBC-velocity was measured in the capillaries contained in three of the nine windows at a magnification of $\times$ 740. Due to this selection, the data of capillary velocities cannot directly be related to the data of FCD, which are obtained from all nine windows. Since the non-perfused capillaries are not visualized by FITC Dextran 150, the mean of the RBC-velocity values is calculated only from perfused vessels.

To better visualize the microvasculature, contrast enhancement was provided prior to ischemia by transillumination using a 443-nm narrow band filter and/or epi-illumination using TRITC-labeled Dextran 70, while FITC Dextran 150 was used for the measurements in the postischemic period. The quality of the image enhanced with FITC Dextran 150 was superior to the one obtained with TRITC Dextran 70, the difference in quality being attributable to the lower permeation of the larger tracer molecules.

Mechanisms of Post-ischemic Reperfusion Failure

The severity of reperfusion injury correlates with the duration of ischemia [48, 49].

Twenty years ago intravascular red cell aggregation [28] and disseminated intravascular coagulation [21] were thought to be responsible for reperfusion failure. At present, destructive effects of oxygen-derived free radicals [19, 35, 44], plugging of capillaries by white cells [2], leukocyte-endothelium interaction [5, 12], capillary endothelial swelling [18, 45], and rheologic abnormalities impairing microflow [31, 50] are discussed as causes of reperfusion failure. It should be noted that several of these factors are at work simultaneously after ischemia. It is well established, that ischemia leads to a shift of ions and water from plasma into the endothelium due to a deficit of energy, required for active separation of cations across the cell membrane. In keeping with this hypothesis, not only an increase in capillary hematocrit, but also swelling of endothelial cells should be expected. To achieve reperfusion at high hematocrit and increased capillary resistance to flow, a high arterio-venous pressure gradient would be necessary to overcome the high-yield shear stress with flow cessation [52].

In the studies presented, 4 h of pressure-induced ischemia in skeletal muscle resulted in severe reperfusion injury. Within the microcirculation the capillary

bed was the most vulnerable part. The small number of reperfused capillaries (only 30%) was a clear indication of no reflow in the capillary bed. The local distribution of FCD became heterogeneous; this, together with the decrease of capillary RBC-velocity, resulted in an additional impairment of the microhemodynamics in postischemic tissue. Similar findings have been obtained by other authors in skin and connective tissue [47, 53], skeletal muscle [20, 25, 53], liver [25], and brain [25].

Effects of Hemodilution in Normal and Ischemic Tissue

Limited intentional hemodilution leads to an improvement of flow conditions and flow properties of the blood without impairment of tissue oxygen supply, despite the decrease of the oxygen carrying capacity [39]. On the contrary, due to the increase in total flow rate, systemic oxygen transport capacity increases with falling hematocrit, reaching a peak value at a hematocrit of about 30%. This was first demonstrated by Hint [24] based on theoretical considerations and has been corroborated by findings from experimental studies [37, 38, 42]. Recently, Mirhashemi et al. [41] reported that even though the saturation of blood at the beginning of the "nutritional" capillary network is only 50% of the systemic value as a result of arteriolar oxygen diffusion and arterio-venous shunting, the amount of oxygen brought to the tissues increases by 14% upon hemodilution to a hematocrit of 30%. Furthermore, in skeletal muscle intentional hemodilution results in a redistribution of blood from the vessels in the connective tissue to the nutritional capillaries with the consequence of enhanced capillary flow [33, 56].

In our experiments isovolemic hemodilution increased FCD but there was no increase in capillary flow velocity. However, the RBC-velocities in the single capillaries were more heterogeneously distributed as compared to reperfusion without preceding hemodilution. Similar results were obtained by Driessen et al. [8] in the mesentery and Baldinger [4] in skeletal muscle. Fritzsche et al. [16] found a more heterogeneous distribution of microvascular hematocrits in the rat mesentery, which was explained by an increased imbalance between red cell and volume flow as a result of hemodilution.

Under normal conditions the capillary hematocrit is about 30% of the systemic hematocrit; therefore, the viscosity of blood approaches the viscosity of plasma. For this reason, hemodilution has little potential to enhance the flow properties within the capillaries, unless hemodilution is associated with a reduction of plasma viscosity. A higher arterio-venous pressure gradient along the microcirculatory bed, however, can improve capillary perfusion by enhancing the functional capillary density, as was observed in our studies. With regard to recruitment of capillaries within the skeletal muscle, Renkin et al. [46] reported, that at rest only 74% of capillaries in rabbit muscle are perfused over 90 s. Similar results have been demonstrated by other authors [7, 32] for different tissues, all studies providing evidence for the presence of a few capillaries presenting without moving RBCs.

In contrast, Oude Vrielink et al. [43] demonstrated that in resting rabbit skeletal muscle all the capillaries are perfused. Kayar and Banchero [26] reported

similar results and furthermore reported a time dependency of capillary perfusion. In the time range of 10 s only 45% of the total number of capillaries in skeletal muscle was perfused. Recruitment was achieved within 30 s. This indicates intermittent capillary blood flow as described previously by Renkin et al. [46].

Improvement of capillary blood flow, elicited by hemodilution, can be achieved by recruitment of a small fraction of capillaries but more efficiently by an increase in flow motion [41]. Improvement of microcirculatory perfusion in ischemia, as a result of hemodilution, has been observed in a number of tissues [23, 54]. Whereas the reduction of the hematocrit to 30% might increase oxygen delivery by 5%–14% in normal tissues, an increase in oxygen delivery up to 66% can be expected in ischemic tissues due to less arteriolar oxygen diffusion and less arterio-venous shunting of oxygen [41].

In the present study, homogeneous distribution of perfused capillaries was preserved during the reperfusion phase by means of prophylactic hemodilution. The initial decrease of RBC-velocity in the capillaries and collecting venules was followed by complete restitution of flow velocities. Fischer and Ames [14] attributed the absence of no reflow after prolonged ischemia to the higher perfusion pressure established with intentional hemodilution. Since hemodilution with Dextran 60 is associated with a marked reduction of blood viscosity at low shear, improvement of capillary flow during the postischemic reperfusion can partly be attributed to facilitated outflow from the capillaries as result of lowered blood viscosity in the postcapillary venules. Further studies are needed to differentiate between dilution vs diluent specific effects.

Clinical Consequences

Perioperative isovolemic hemodilution has established itself in surgical practice as a means of reducing the number of transfusions of homologous blood and to avoid the side-effects inherent to transfusion. Recent reports in the literature regarding preoperative and intraoperative hemodilution confirm that hematocrits of 27%–30% are well tolerated by patients undergoing general surgery, thoracic surgery, vascular, coronary and open heart surgery, neurosurgery, and orthopedic surgery in adults and children (for review see [36]). A low perioperative hematocrit not only minimizes the risk of specific complications, such as deep venous thrombosis, pulmonary emboli, myocardial infarction and cerebral vascular insults, but reduces at the same time the overall incidence of postoperative complications, including wound healing problems [3, 6].

Of even greater importance, however, are the hemodilution-induced changes of the macro- and microhemodynamics in ischemia. In 1956, Gelin [17] demonstrated in his "studies on anemia of injury" that under conditions of impaired blood fluidity and consecutive microcirculatory perfusion failure the stagnant blood undergoes spontaneous dilution by influx of interstitial fluid. Low hematocrit results in a lower yield shear stress, thus facilitating postischemic reperfusion. In patients with low hemoglobin levels a decrease in the frequency of postoperative reocclusion following arterial reconstruction in ischemic limbs has been reported [34]. Furthermore, a close correlation between preopera-

tively normal or low hemoglobin levels and outcome of distal amputation has been demonstrated in diabetic patients [3].

These findings suggest that hemodilution should be implemented into the prophylactic and therapeutic strategies for salvaging marginally ischemic tissue.

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