Microcirculatory damage of the periosteum
From clinical case history to animal experiments and back to the bedside

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1. BACKGROUND

Direct and indirect trauma forces leading to fractures usually involve injury to the periosteum, the outer covering layer of the skeleton. Stability and controlled micro-motions of the bones are very important components of trauma therapies, but the biological response of the periosteal sheet is also an essential factor in the repair mechanism of the bony architecture. Indeed, it has been shown that the periosteum not only protects and feeds the bone cortex, but also strongly influences osteogenesis.

Apart from traumas, periosteal damage can be a consequence of clinical interventions. Various emergency and elective orthopedic operations are performed under artificially reduced blood flow conditions, leading to regional ischemia differing in degree and duration. As an example, vascularized bone autografts are frequently used in reconstructive surgery for the replacement of large bone defects. Harmful consequences of the re-establishment of the vascular supply have often been observed after technically successful replantations or free flap transplantations. Today it is well established that the primary ischemic damage (when the bloodless period exceeds the critical tolerance duration) is significantly aggravated by secondary events during reperfusion. The ischemia-reperfusion (I-R) phenomenon includes various pathophysiological cascade mechanisms which enhance local and remote tissue injuries. Tourniquet methods *per se* are I-R events, which can cause healing disorders, including delayed bone healing, pseudoarthrosis or sequester formation.

Apart from local occlusion-induced circulatory derangements, similar I-R events may occur in some type of trauma events can lead relatively frequently to avascular bone necrosis. This is a time- and region-dependent ischemic injury resulting from temporary or permanent loss of the blood perfusion to the bones. Although this can occur in any bone, avasular necrosis most commonly affects the ends of long bones such as the femur, after hip joint dislocation. When joints are injured, blood vessels may be damaged. The increase in intraarticular pressure causes the blood vessels to narrow, making it difficult for them to deliver enough blood to the bone cells. Repair of the damage to the circulation is generally achieved spontaneously within a few hours after the closed repositioning of the joint.

However, there are special sites where the risk of avascular necrosis is very high, *e.g.* the peritalar region. The talus is the only bone in the lower extremity without any muscular attachments, making it somewhat vulnerable in the event of injury. Talus fractures can often be accompanied by partial or total talus necrosis. Most talus body or neck fractures are observed relatively frequently and this injury can also accompany supra- or subtalar sprains. The specific mechanism of this injury has not been described. It is thought that total talar

dislocation is the endpoint of maximum pronation or supination injuries, the final result of a continuum of forces that begin with dislocation of the subtalar joint. Injuries can occur after falling from a height or in motor vehicle accidents.

The all types of I-R phenomenon can influence essentially the microperfusion of the periosteum and the bone. The microcirculation in general can be analyzed *in vivo* by intravital microscopy, a dynamic technique used to observe and quantify the microcirculatory alterations in well-defined structures in living tissues. However, as opposed to other organs, characterization of the human bone or periosteal microcirculation is still incomplete. Some observations have suggested that the periosteal microcirculation may be a good indicator of the perfusion changes of the whole bone, but the exact microcirculatory components involved in the pathogenesis of skeletal I-R remain unknown, mainly because of methodological limitations.

The few animal studies that have been reported have focused mainly on the effects of a flow reduction or soft tissue trauma-induced local microcirculatory reactions of the periosteal microcirculation. For this reason, there is a clear need for the investigation and characterization of periosteal microcirculatory reactions in animal models of clinical I-R conditions, such as bone autotransplantation or tourniquet ischemia.

As concerns trauma care, elimination of the factors obstructing the bone and periosteal microcirculation is the primary goal. The following important step is the use of pharmacological treatment to improve the blood supply to the injured region. In line with this, effective fluid therapy is of the utmost importance in critically ill patients with trauma-associated injury and blood loss, in order to restore the impaired tissue perfusion. The administration of intravenous fluid is indicated to avoid dehydration, to maintain an effective circulating volume, and to prevent inadequate tissue perfusion. These aims should all be considered core elements of perioperative practice. Attempts to achieve this goal are currently made by the administration of a variety of crystalloid and colloid solutions or an infusion of their combinations. The global circulatory effects of colloid or crystalloids fluid therapy have been examined in several studies, but their influences on the periosteum have not been analyzed yet.

On the basis of this background, this thesis focuses on the recognition of periosteal microcirculatory damage and its importance in the clinical practice of traumatology. Analysis of the experimental post-ischemic changes in the periosteal microcirculation could provide important information via which to improve our knowledge not only on the complications of

fractures or bone autotransplantation, but also on other trauma cases accompanied by temporary bloodless conditions.

1.1. Microcirculatory consequences of ischemia-reperfusion injury

I-R initiates a cascade of pathophysiological events which in turn enhance the local and remote tissue injury. With severe blood flow deficits and impaired oxygen consumption, the oxidative phosphorylation and metabolic functions are deranged. I-R injury has immediate and local effects, and there is substantial evidence that disturbances of the local microcirculation are involved in this syndrome. Organ hypoperfusion and reperfusion generate a local inflammatory environment that primes circulating leukocytes which provoke distant organ injury.

The most convincing data concerning the consequence of this reaction derive from intravital microscopy studies. The technology allows real-time imaging of the microcirculation and the exact determination of the consequences of I-R. Disturbances of the microcirculatory perfusion are characterized by changes in the functional capillary density (FCD) and the red blood cell velocity (RBCV, $\mu m\ s^{-1}$). The FCD is defined as the length of red cell-perfused capillaries in relation to the observation area, which accurately describes the decrease in the efficacy of tissue perfusion when the corresponding area is unchanged. The RBCV is determined primarily by the blood flow and the cross-section of the circulatory area. The main causes of microcirculatory disturbances are as follows:

1. Changes in perfusion and vasoactivity

Microcirculatory dysfunctions seem to be mediated by endothelial cell damage and an imbalance of vasoconstrictor and vasodilator molecules, such as endothelin, reactive oxygen species and nitric oxide. The hypoxia-induced extensive release of vasoconstrictor mediators can lead to significant vasoconstriction of the precapillary sphincters, *i.e.* a considerable proportion of the inflowing blood returns to the venules without passing the capillaries. Precapillary vasoconstriction can also account for the relatively small decrease in arteriolar RBCV, which is determined primarily by the blood flow and the cross-section of the circulatory area. An aggravating consequence of I-R, the no-reflow phenomenon, may develop as a result of interstitial edema formation and external compression of the capillaries, or it may be a result of intraluminal plug formation.

2. Cellular activity: leukocyte-endothelial interaction

Investigations utilizing intravital microscopy have demonstrated that the recruitment of inflammatory cells into the perivascular tissue involves a complex cascade mechanism. The

adhesion process consists of several steps, beginning with the rolling of the polymorphonuclear leukocytes (PMNs) on the endothelial surface of the postcapillary venules until they have slowed down to such a degree that they stick to the endothelium. At this point, the leukocytes are sequestered from the main vascular flow, and firm adherence to the endothelial cells may follow. Subsequently, the leukocytes pass an intercellular junction between the endothelial cells and reach the abluminal side.

As opposed to other organs, the periosteal changes in response to I-R have been only poorly characterized. The few studies examining the periosteal microcirculation have focused on the effects of flow reduction or the soft tissue trauma-induced local microcirculatory reactions.

1.2. Significance of volume therapy in circulatory disturbances

Absolute or relative blood volume deficits are often observed in the surgical, trauma or intensive care patient. The administration of intravenous fluid to avoid dehydration, maintain an effective circulating volume and prevent inadequate tissue perfusion should be considered a core element of perioperative practice. The overall goal of fluid therapy is to ensure an adequate oxygen supply for the organs. However, there is an ongoing debate with regard to the choice of the optimal type and timing of fluid resuscitation and the most appropriate endpoint of resuscitation. Besides trauma-associated blood loss in patients at the scene, effective fluid therapy is of utmost importance in critically ill patients with circulatory instability.

Research is ongoing to define the ideal fluid for trauma and hemorrhage and for intraoperative volume support. In general, crystalloids and colloids, with either isotonic/normoncotic or hypertonic/hyperoncotic modifications, and also blood, blood substitutes and oxygen therapeutics, are available. Resuscitation with isotonic (270-310 mosmol l^{-1}) crystalloid solutions, *i.e.* lactated Ringer's (LR) solution or normal (0.9%) saline, is the current standard and predominates over the use of all other fluids for resuscitation. Crystalloids are distributed throughout the body and primarily fill the interstitial space without any preference for the intravascular space. Thus, the plasma-expanding effect is poor and is considered to be only 10-20%. However, fluid resuscitation with crystalloids can enhance interstitial edema, in consequence of the reperfusion injury to the capillary interstitial membrane.

When higher volume expansion is sought, when concerns over edema formation arise, or when the patient responds only transiently and even remains hypotensive, crystalloids are

replaced by colloids, or colloids are added. In general, solutions of dextran (DEX), albumin (ALB) gelatin (GEL) are available and hydroxyethyl starch (HES) being the most common artificial colloid used. By virtue of their water-binding capacity, colloids prolong the circulatory effect by retaining water in the vascular space.

The volume-restoring potential of colloids is often regarded as a most important clinical feature; nevertheless, the net efficacy of 'plasma expanders' is determined by many other factors. Specifically, the various experimental and clinical data demonstrate significant differences not only in the characteristics of the macrohemodynamic responses, but also in the potential to restore tissue oxygenation and additionally the potential to modulate inflammatory activation at the microcirculatory level. Anti-inflammatory actions and microcirculatory consequences are in fact interrelated events. Interruption of the adhesion between PMNs and endothelial cells ameliorates or prevents microcirculatory dysfunctions, and these reactions have been implicated as critical pathogenetic factors in a variety of low flow-induced tissue injuries.

Our understanding of the pivotal role of activated PMNs in the pathogenesis of a microvascular dysfunction raises questions concerning the opportunities for fluid therapy in the prevention or treatment of this syndrome. To date, however, there have been only very few *in vivo* studies where the microcirculatory effects of the clinically most important artificial colloid classes, including DEX, low molecular weight (MW) HES and GEL, have been characterized and compared in the same setup.

2. MAIN GOALS

The main goals of the present studies were:

1. To demonstrate the significance of the duration of ischemia as concerns the recovery of a peritalar sprain through a human case history.
2. To design an experimental rat model of complete limb ischemia in order to elucidate the periosteal microcirculatory alterations caused by I-R. This included examinations of the efficacy of the tissue perfusion and the primary and secondary leukocyte-endothelial cell interactions.
3. A further aim was to characterize the effects of DEX (6%; 60 kDa MW), low MW HES (6%; 130 kDa/0.4) and GEL (4%; 35 kDa) on PMN reactions by using standardized *in vivo* microscopic methods to demonstrate their relative therapeutic benefits in ameliorating I-R-induced microcirculatory disturbances.

3. MATERIALS AND METHODS

3.1. Human clinical study: medical attendance of injury of the peritalar region. A case history

A 23-year-old sportswoman suffered a peritalar sprain (a complete talus sprain) in her right leg, while participating in a long jump rally. Since the sports-ground was located near the place of treatment (a trauma care unit), following quick transport, the injury was diagnosed only a few minutes after the event. During transport, the complete sprain of the talocrural joint predominated in the clinical situation and the bones of the ankle almost perforated the skin medially (from the inside). Blood supply disturbances were clearly recognizable in this region. Immediate repositioning was unsuccessful. Temporarily, a Kramer rail was fixed on the injured limb, following which a radiological examination was performed. The X-ray revealed a sprain of the subtalar region and subluxation of the talus in the talocrural joint. Closed repositioning of the sprain of the calcaneonavicular joint was subsequently carried out under anesthesia. After successful repositioning, a lower shank split concentric plaster was applied.

3.2. Animal experiments

The experiments were performed in two main series on male Wistar rats (average weight 300±35 g). The rats were anesthetized with sodium pentobarbital (45 mg kg^{-1} ip) and the trachea was cannulated to facilitate respiration. The right jugular vein and carotid artery were cannulated for fluid and drug administration and for the measurement of arterial pressure, respectively. To determine cardiac output (CO) changes, the left common carotid artery was also dissected and a thermistor-tip catheter was introduced into the ascending aorta to measure the CO by a thermodilution technique. An ultrasonic flow probe was placed around the exposed femoral artery to measure the blood flow.

The animals were placed in a supine position on a heating pad to maintain the body temperature between 36 and 37 ^{o}C. The periosteum of the medial surface of the right tibia was exposed under a Zeiss 6x magnification operating microscope. By means of an atraumatic surgical technique, the skin above the anterior tibia was dissected and the gracilis posterior muscle was cut through. This simple, novel, easily reproducible procedure provides a tissue window with good exposure of the proximal and medial microvascular architecture of the anterior tibial periosteum without using local microcirculatory disturbances or inflammatory reactions.

The right hindlimb with the exposed tibia was positioned horizontally on an adjustable

stage and superfused with 37 ^{o}C saline. The microcirculation of the distal tibia was visualized by intravital microscopy (Zeiss Axiotech Vario 100HD microscope, 100 W HBO mercury lamp, Acroplan 20x water immersion objective), using fluorescein isothiocyanate labeled erythrocytes for red blood cell staining and rhodamine-6G staining for leukocytes. The microscopic images were recorded with a charge-coupled device videocamera (AVT HORN-BC 12) attached to an S-VHS videorecorder (Panasonic AG-MD 830) and a personal computer.

Quantitative assessment of the microcirculatory parameters was performed off-line by frame-to-frame analysis of the videotaped images, using image analysis software (IVM, Pictron Ltd., Budapest, Hungary). The FCD, *i.e*. the length of the perfused nutritive capillaries per observation area (cm^{-1}), and the RBCV ($\mu m\ s^{-1}$) were measured in 5 separate fields in 5 capillaries at each time point of each experiment. Leukocyte-endothelial cell interactions were analyzed within 5 postcapillary venules (diameter between 11 and 20 μm) per animal. Adherent leukocytes (stickers) were defined in each vessel segment as cells that did not move or detach from the endothelial lining within an observation period of 30 s, and are given as the number of cells per mm^2 of endothelial surface. Rolling leukocytes were defined as cells moving at a velocity less than 40% of that of the erythrocytes in the centerline of the microvessel, and are given as a percentage of the number of nonadherent leukocytes passing through the observed vessel segment within 30 s.

3.2.1. Experimental protocols

In the first series of experiments (Study I), I-R-induced microcirculatory changes in the tibial periosteum were analyzed with the aid of fluorescence intravital microscopy. After a 30-min stabilization period, the baseline cardiovascular and microhemodynamic parameters were determined (baseline; t = -60 min). The animals were allotted into one or other of two experimental groups. The first group (n=5) served as sham-operated controls to exclude microcirculatory changes relating solely to the anesthesia and surgery. In group 2 (n=5), complete hindlimb ischemia was induced by clamping the femoral artery with an atraumatic vascular miniclip and placing a tourniquet around the femur, immediately after the occlusion of the vessel. After ischemia for 60 min, the tourniquet and the artery clip were removed, and the reperfusion was observed for 180 min. The periosteal microcirculation was observed hourly during the 180-min reperfusion period.

In the second series of experiments (Study II), the effects of volume resuscitation with crystalloid and different colloid solutions on the I-R-related microcirculatory disturbances of

the periosteum were examined. In these experiments, LR solution was infused at a rate of 10 ml kg^{-1} h^{-1} during the surgical procedures and for 50 min during the ischemic phase of the experiments. The first group (*n*=10) served as LR-treated, sham-operated controls, where the microcirculatory variables were recorded for 240 min to exclude changes relating solely to the anesthesia and surgery. In the next four groups, complete hindlimb ischemia was induced by placing a tourniquet around the proximal femur, with simultaneous occlusion of the femoral artery with a miniclip for 60 min. The occlusions were then released (t = 0 min), and the periosteal microcirculation was observed after reperfusion for 180 min. The animals in groups 2-4 were treated with LR (*n*=10), GEL (Gelofusine 4%; 35 kDa; *n*=6), DEX (Macrodex 6%; 60 kDa; *n*=9) or HES (Voluven 6%; 130 kDa/0.4; *n*=8), respectively, in a dose of 15 ml kg^{-1} h^{-1} iv, starting during the last 10 min of ischemia. The infusions were maintained during the first hour of reperfusion and the doses were then decreased to 5 ml kg^{-1} h^{-1} iv in the second and third hours of reperfusion.

3.2.2. Statistical analysis

Data analysis was performed with a statistical software package (SigmaStat for Windows, Jandel Scientific, Erkrath, Germany). Nonparametric methods were used. Friedman repeated measures analysis of variance on ranks was applied within the groups. Time-dependent differences from the baseline were assessed by Dunn's method. Differences between groups were analyzed with Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method for pairwise multiple comparison.

4. RESULTS

4.1. Human study

As a result of resting for a few days with suspended extremities and diuretic therapy, the initial edema disappeared, and on day 6 the initial split plaster fixing could be replaced by a closed concentric plaster. During physiotherapy, the patient was taught to walk with crutches, without using her injured leg. At the control examination in the 6th week, the plaster binding could be removed. The clinical examination showed stability of the talocrural joint. The X-ray demonstrated congruity on the surface of the joints and verified the stability of the joints. However, the complementary MR record demonstrated a localized, intraosseal circulatory disturbance. At this time, for amelioration of the blood supply and joint motion, cautious physiotherapy was started (active/passive gymnastics) with continued strict loading prohibition. This led to the range of motion of the patient improvings and the elimination of pain in the course of time. After the 16th week, MR examination showed revascularization at

the site of the earlier circulatory disturbance in the talus. Clinical examination indicated the same range/extent of motion as on the intact side. In view of the favorable examination results, partial (and after the 20th week full loading of the extremity was allowed. After the 20th week, the patient was completely pain-free. The annual control showed the perfect healing of the affected bone parts.

4.2. Experimental study I. Effects of I-R on postischemic periosteal microcirculatory changes

Intravital microscopy revealed homogenous microvascular perfusion in the periosteum in both groups under the baseline conditions. The RBCV was similar in both groups and did not change over time in the sham-operated group. I-R, however, led to a significantly decreased RBCV during the reperfusion period. The periosteal FCD did not change significantly in the sham-operated group. However, I-R caused a significant decrease in FCD, which decreased gradually from the beginning of the reperfusion and reached the lowest value (60% of the baseline) at 120 min of reperfusion.

Leukocyte-endothelial cell interactions were characterized by the number of rolling and adherent (stickers) leukocytes. A maximum of 30% of the nonadherent PMNs rolled along the endothelial lining of the postcapillary venules under the baseline conditions in the different groups. In the sham-operated control group, there were no significant changes in the numbers of rolling and adherent PMNs at any of the observation points throughout the experiments. The 60-min ischemia and reperfusion was accompanied by a significant increase in leukocyte-endothelial cell interactions. Both the percentage of rolling cells and the number of adherent PMNs were increased as compared with the preischemic values or the values for the sham-operated group at matching time points.

4.3. Experimental study II. Effects of colloid solutions on the consequences of I-R

The CO and femoral artery blood flow were measured to characterize the peripheral and local hindlimb perfusions. There was a nonsignificant (approximately 14-22%) tendency to a decreased CO at the end of the ischemic period in all the ischemic groups. The volume replacement therapies normalized the CO in all the groups, and there were no significant differences in these values within or between the groups during the reperfusion period. The femoral artery blood flow increased transiently during the first hour of reperfusion in all the I-R groups. Although the highest flow increase was observed in the HES-treated animals, this change was again statistically not significantly different between the treated groups during the reperfusion period. The colloid infusion-caused changes in volume expansion were assessed

by hematocrit measurement. As compared with the baseline values, there was an increase in hematocrit at the end of the ischemia (before volume replacement) in all the ischemic groups, but this was followed by a complete restoration to the baseline values in the later experimental phase.

Intravital microscopy revealed homogenous microvascular perfusion in the periosteum in all groups under the baseline conditions. In the LR-treated group, however, I-R led to a significant RBCV decrease (by 39%) during reperfusion. The microcirculatory perfusion did not improve in response to GEL; in this group, lower RBCV values were observed than in the sham-operated group or under the baseline conditions. After a transient restoration during reperfusion, DEX treatment did not influence the RBCV changes induced by ischemia. HES treatment prevented the postischemic deterioration of RBCV nearly completely, and a complete restoration to the baseline values was observed after the first hour of reperfusion.

In the sham-operated group, the periosteal FCD did not change significantly, but was greatly and permanently deteriorated in the LR-treated I-R group after 60 min of reperfusion. The GEL treatment caused a transient postischemic improvement, but this was followed by a progressive deterioration after 60 min of reperfusion. DEX infusion did not lead to any amelioration of this parameter throughout the entire reperfusion phase. However, a considerable protection against I-R-induced capillary perfusion failure was provided by HES, which caused a complete restoration of the FCD, similar to that seen under the sham-operated conditions. This difference was statistically significant as compared with the effects of LR, DEX and GEL after I-R.

In the sham-operated group, the numbers of firmly adherent leukocytes did not change significantly during the experiments. In the I-R group, the proportion of rolling leukocytes increased from a baseline level of approximately 16% to around 37% and 43% after 120 min and 180 min of reperfusion, respectively. Significant increases were observed in the number of sticking leukocytes at 120 min of reperfusion. The postischemic increases in the proportion of rolling leukocytes were not influenced by any of the colloid treatments, though the HES infusion caused a moderate improvement in the later stages of reperfusion. However, the I-R-induced firm leukocyte adherence was completely prevented by HES throughout the entire reperfusion period. In contrast, significant deteriorations in this parameter were observed in the first hour of reperfusion in the GEL- and DEX-treated groups, similarly to that seen after I-R with LR treatment. In the case of GEL treatment, this was followed by a moderate improvement in the later experimental phase.

5. DISCUSSION

5.1. Human study

Traumatological events lead relatively frequently to avascular necrosis, which is a time- and bone region-dependent ischemic injury. However, the peritalar region is of special interest, because here the risk of avascular necrosis is also very high. The talus is the only bone in the lower extremity without any muscular attachments, making it somewhat vulnerable in the presence of injury. Furthermore, talus fractures are often accompanied by partial or total talus necrosis. Total dislocation of the talus (peritalar dislocation) is uncommon, accounting for approximately 2% of all traumatic talus injuries. The majority of these injuries are accompanied by fracture of the talus (occurring mainly as an open fracture). Total dislocation of the talus has a 90% probability of a poor prognosis and osteonecrosis. The specific mechanism of this injury has not been described. It is thought that total talar dislocation is the endpoint of maximum pronation or supination injuries, the final result of a continuum of forces that begin with dislocation of the subtalar joint. Injuries can occur after falling from a height or in motor vehicle accidents.

The clinical case reported here demonstrated a very rare peritalar dislocation, where neither partial nor complete necrosis of the talus developed. The management strategy was based on the early diagnosis and MR documentation. Additionally, the following factors could play a pivotal role in the absence of complications:

- Repositioning could be performed quickly, owing to the rapid transport between the trauma care unit and the scene of the accident.
- The duration of the plaster cast fixation applied to maintain the result of the repositioning was as short as possible so as to avoid soft tissue damage, it lasted only until the ligament stability of the affected joints was achieved.
- Non-weight-bearing active and passive physiotherapy had a beneficial effect on the impairment of the intraosseal circulation detected subsequently to the plaster cast fixation. After the recovery of the blood supply of the talus was demonstrated by the control MR examination, weight-bearing mobilization of the extremity could begin. The application of MR tomography in the diagnosis and the therapy of this injury was highly advantageous.

In summary, appropriate diagnosis and documentation of the circulatory state by means of MR tomography, and early treatment of the ischemic injury, resulted in the most advantageous management.

5.2. Experimental study I

In these experiments, we employed a rat tibia model to investigate the periosteal microcirculatory consequences of a standardized I-R challenge. Although complete vascular occlusion disturbs the perfusion of all the tissues of the exposed limb, microcirculatory deterioration predominates in the periosteum, while the surrounding muscle layers exhibit much lower ischemic sensitivity.

Our results demonstrated that a 60-min period of ischemia induces capillary perfusion failure and leukocyte-endothelial cell interactions in the periosteal microcirculation. During the reperfusion phase, the proportion of perfused capillaries decreased significantly, and thus a large proportion of the inflowing blood returned to the venules without passing the capillaries. The reason for this shunt circulation may be precapillary vasoconstriction, but other reperfusion-related factors can also contribute to the reduction of the FCD. Additionally, we have observed increased leukocyte-endothelial cell interactions in the postcapillary venules. In the periosteal vessels with smaller diameter, the adherent and rolling leukocytes form typical leukocyte plugs, thereby probably leading to obstruction of the venules. The adhesion process consists of several steps, beginning with the rolling of the PMNs on the endothelial surface of the postcapillary venules until they have slowed down to such a degree that they stick to the endothelium. As the enhanced leukocyte-endothelial cell interactions eventually lead to leukocyte extravasation, this process plays an important role in reperfusion-associated late tissue injury too.

5.3. Experimental study II

This study investigated the anti-inflammatory actions of major artificial colloid classes in the tibial periosteal microcirculation, by comparing their effects on the perfusion characteristics and the leukocyte-endothelial interactions. In this tissue compartment, the significantly reduced FCD and capillary RBCV were accompanied by characteristic leukocyte-mediated reactions in the reperfusion phase. Of the examined solutions, these potentially harmful changes were abolished only by the HES infusion.

The volume-expanding properties of the three colloid solutions might differ due to their differing MWs and vascular retentions, but the macrohemodynamic changes suggested quite similar volume-related effects for the examined solutions. The matching macrocirculatory endpoints indicated that other parameters, including microcirculatory changes, could be accurately compared in this setup.

We have presented evidence that PMN sticking is inhibited by HES throughout the entire reperfusion phase, and HES interferes significantly with the PMN-derived CD11b integrin expression, whereas the other colloids did not affect this parameter. This finding might refer to a situation where various doses of HES inhibit the tissue nuclear factor-κB activation and systemic tumor necrosis factor-α elevation after local and systemic inflammatory insults.

It has also been suggested that colloid solutions affect PMN adherence directly, by influencing the adhesion molecule expression independently of microhemodynamic changes. However, microhemodynamics and leukocyte adhesion are interrelated phenomena. Not only endothelium-derived vasoactive substances (typically nitric oxide and ET-1), but also perfusion changes and secondary shear stress *per se* influence PMN activation and adhesive interactions by changing the levels of expressions of adhesion molecules or the dynamics and half-lives of molecular bonds. It has been shown that the reduced velocity of PMNs near the vessel wall increases the probability of PMN adhesion and, likewise, an increased shear rate can reduce the number of adherent cells. Specifically, a force below the shear optimum can induce an increase in PMN rolling, while an increased wall shear stress enhances the velocity of rolling leukocytes and consequently decreases adhesion. In the case of HES, nearly complete restoration of the baseline RBCV was observed during reperfusion, whereas the other colloids were ineffective. These observations might be indicative of an indirect, flow- or volume-dependent anti-inflammatory effect, as this improvement could affect the perfusion of the postcapillary venules and thereby also contribute to the reduction of PMN-endothelial interactions.

In conclusion, the minimization of the ischemic duration and quick restoration of the injured periosteal circulation can promote the avoidance of avascular necrosis in cases of an extreme peritalar sprain. In addition, this special case history embedded into this thesis suggests that the application of animal models for the clarification of human clinical problems is of enormous benefit. Our rat model allowed *in vivo* visualization of the microcirculation of the rat tibial periosteum in an experimental setting that aimed at simulating the clinical situation of vascularized bone autotransplantation. The beneficial effects of HES on the microvascular perfusion were paralleled by local anti-inflammatory actions and the prevention of systemic PMN activation, whereas the other colloid solutions did not influence these parameters. Although any extrapolation to clinical applications should be attempted only with caution, it appears reasonable to suggest that isovolemic hemodilution with HES provides a therapeutic advantage in this setting.

6. SUMMARY OF NEW FINDINGS

1. Our *in vivo* experiments permitted quantification of the microcirculatory alterations caused by limb I-R in a clinically relevant animal model.
2. The I-R injury was manifested in a deterioration of the efficacy of the periosteal microvascular perfusion and the PMN-endothelial interactions are critically linked to the microcirculatory derangement in the reperfused tissues
3. The different colloid solutions exert diverse microcirculatory effects after limb ischemia. Only HES displayed a significant alleviating effect locally and in the prevention of systemic PMN activation, whereas the other colloid solutions (DEX and GEL) did not induce similar protection. This underlines the advantages of the use of HES in the care after trauma surgery.

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