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Limb ischemia–reperfusion differentially affects the periosteal and synovial microcirculation

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ABSTRACT

Background: Joints are privileged compartments that enjoy increased protection against the inflammatory reactions affecting the extremities. We hypothesized that the functional characteristics of the microvasculature would contribute to the differential defensive potential of the synovial membrane.

Methods: We investigated the synovial microcirculatory reactions and compared them with those of the tibial periosteum in response to 60 min of total limb ischemia, followed by 180 min of ischemia–reperfusion (IR) in rats. Carrageenan/kaolin-induced knee monoarthritis, a neutrophil-driven synovial inflammation model, served as the positive control.

Results: IR brought about a significant reduction in red blood cell velocity in the capillaries and increases in rolling and adherence of the neutrophil leukocytes in the postcapillary venules (intravital microscopy), in adhesion molecule expression (intercellular adhesion molecule-1 immunohistochemistry) and in xanthine oxidoreductase activity in the periosteum. These changes were also pronounced in carrageenan/kaolin-induced monoarthritis but were almost completely absent in the synovium after the IR challenge. Most importantly, even after IR and in carrageenan/kaolin monoarthritis, the synovial microcirculation was characterized by significantly greater red blood cell velocities than that in the periosteum under resting conditions.

Conclusions: The ischemic duration, which significantly affected the functional integrity of the periosteal microcirculation, did not bring about a marked deterioration in that of the synovial membrane, suggesting that the synovial microcirculation is less endangered to the consequences of short-term tourniquet exposure than the periosteum. The greater microcirculatory red blood cell velocities and lower IR-induced endothelial expression of intercellular adhesion molecule-1 in the synovial membrane might explain the greater resistance of this compartment to the inflammatory consequences of IR.

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1. Introduction

Knee surgery because of orthopedic trauma often requires bloodless conditions (i.e., arthroscopy or arthroplasty), which are usually elicited by tourniquet application. Although the application of a tourniquet offers several benefits such as reduced intraoperative blood loss [1], better cementation owing to a clearer operative field, and a possible reduction in the duration of the operation, certain complications have also been reported [2]. These difficulties include damage to vessels (deep venous thrombosis and edema), injuries affecting the soft tissues, muscles, and nerves (neurapraxia and paralysis), and increased swelling of the joints after release of the tourniquet. Some of these complications originate from the direct mechanical forces elicited by the tourniquet application itself; however, biochemical consequences of vascular occlusion/reperfusion might also have a major effect in the pathogenesis of these clinical symptoms [3]. In such cases, the reestablishment of perfusion subjects the skeletal system to ischemia–reperfusion (IR), and the subsequent oxidoreductive stress leads to inflammatory reactions [4,5]. Periosteal microcirculatory dysfunction and evolving leukocyte–endothelial interactions are significant components of tourniquet-induced inflammatory activation [6]; however, the magnitude of IR-caused inflammatory changes in the synovium is still unknown. One clear difference between these structures is that the joints are body compartments that are somewhat sheltered from the consequences of systemic noxious stimuli. In antigen-dependent inflammatory disorders, the joints are transiently protected from the consequences of generalized infections [7], and the penetration of certain drugs into the joint cavity is also greatly limited [8–10]. The question, therefore, arises of whether the functional characteristics of the membrane microcirculation contribute to this differential defensive potential. Accordingly, our primary goal was to characterize the parallel microvascular inflammatory reactions of the tibial periosteum and the synovium of the knee joint in response to a standardized IR challenge. With this aim, we used intravital microscopy (IVM) for the *in vivo* visualization of the primary and secondary polymorphonuclear (PMN)–endothelial interactions in the postcapillary venules and the changes in red blood cell velocity (RBCV) in the capillaries of the tibial periosteum and synovium of the knee joint. For examination of the synovial membrane, we used a method that allows direct observations at the inner surface of the joint cavity [11]. Second, we compared these changes with those seen in carrageenan/kaolin (C/K)-induced knee osteoarthritis, a classic model of local sterile inflammation known to be mediated by PMN leukocytes [11,12].

2. Methods

The experiments were performed in accordance with the National Institutes of Health Guidelines (Guide for the Care and Use of Laboratory Animals), and the Animal Welfare Committee of the University of Szeged approved the study.

2.1. Surgical procedure

Male Wistar rats (average weight 300 ± 20 g; $n = 8–11$) were anesthetized with sodium pentobarbital (45 mg · kg^{-1} intraperitoneally), and the right jugular vein and carotid artery were cannulated for fluid and drug administration and the measurement of arterial pressure (a Statham P23Db transducer with a computerized data acquisition system; Experimetria, Budapest, Hungary), respectively. The rats were placed in a supine position on a heating pad to maintain the body temperature at 36°–37°C, and Ringer's lactate was infused at a rate of 10 ml · kg^{-1} · h^{-1} during the experiments, together with small supplementary doses of pentobarbital intravenously, when necessary. The trachea was cannulated to facilitate respiration, the right femoral artery was dissected free, and the periosteum of the medial surface of the right tibia was exposed under a Zeiss 6× magnification operating microscope (Carl Zeiss GmbH, Jena, Germany), using an atraumatic surgical technique [6]. The synovial membrane over the medial condyle of the proximal tibia was exposed, as previously described [11]. In brief, the articular capsule was opened, and the patella was turned gently upward. The synovium rising along the medial condyle of the tibia was visualized on the inner surface of the joint cavity.

2.2. Arthritis induction

The rats were anesthetized with intraperitoneal ketamine (50 mg · kg^{-1}) and xylazine (12 mg · kg^{-1}), the skin over the knee was disinfected with povidone iodide, and arthritis was then induced with a single intra-articular injection of 75 μL of a mixture of 2% λ-carrageenan (Sigma Aldrich, St. Louis, MO) and 4% kaolin in saline [12].

2.3. Experimental protocols

The experiments were performed in 2 major series, with the rats allotted to 1 or other of the following experimental groups. In the first group, the periosteal and synovial microcirculatory responses to 60 min of total limb ischemia followed by a 180-min reperfusion period were examined (IR, $n = 6$). After recording the baseline microcirculatory variables (time = −60 min) with fluorescence IVM, complete hindlimb ischemia was induced by placing a tourniquet around the proximal femur, with simultaneous occlusion of the femoral artery with a miniclip. The occlusions were then released (time = 0 min), and the periosteal and synovial microcirculation were observed using IVM at 180 min in the reperfusion phase. In the second series, arthritis was induced in the anesthetized rats with a single intra-articular injection of 75 μL of the C/K mixture in saline [12]. IVM examinations of the knee joints were started 6 h after arthritis induction [11].

Tissue specimens for immunohistochemical analysis and biochemical determinations were taken at the end of the experiments. The tissue biopsies were fixed in buffered formalin, and biochemical samples were stored at −20°C.