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Use of dextran sulfate in tourniquet-induced skeletal muscle reperfusion injury

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ABSTRACT

Background: Lower extremity ischemia–reperfusion injury (IRI)—prolonged ischemia and the subsequent restoration of circulation—may result from thrombotic occlusion, embolism, trauma, or tourniquet application in surgery. The aim of this study was to assess the effect of low-molecular-weight dextran sulfate (DXS) on skeletal muscle IRI.
Methods: Rats were subjected to 3 h of ischemia and 2 or 24 h of reperfusion. To induce ischemia the femoral artery was clamped and a tourniquet placed under the maintenance of the venous return. DXS was injected systemically 10 min before reperfusion. Muscle and lung tissue samples were analyzed for deposition of immunoglobulin M (IgM), IgG, C1q, C3b/c, fibrin, and expression of vascular endothelial-cadherin and bradykinin receptors b1 and b2.
Results: Antibody deposition in reperfused legs was reduced by DXS after 2 h ($P < 0.001$, IgM and IgG) and 24 h ($P < 0.001$, IgM), C3b/c deposition was reduced in muscle and lung tissue ($P < 0.001$), whereas C1q deposition was reduced only in muscle ($P < 0.05$). DXS reduced fibrin deposits in contralateral legs after 24 h of reperfusion but did not reduce edema in muscle and lung tissue or improve muscle viability. Bradykinin receptor b1 and vascular endothelial-cadherin expression were increased in lung tissue after 24 h of reperfusion in DXS-treated and non-treated rats but bradykinin receptor b2 was not affected by IRI.
Conclusions: In contrast to studies in myocardial infarction, DXS did not reduce IRI in this model. Neither edema formation nor viability was improved, whereas deposition of complement and coagulation components was significantly reduced. Our data suggest that skeletal muscle IRI may not be caused by the complement or coagulation alone, but the kinin system may play an important role.

1. Introduction

Lower extremity ischemia–reperfusion injury (IRI) may result from thrombotic occlusion, embolism, trauma, or surgical intervention requiring tourniquet application to establish a blood-free environment [1]. Although the short-term use of a tourniquet for less than 30 min has no effect on postoperative pain or functional recovery [2], prolonged application can be associated with IRI and considerable pathophysiological alterations including edema, loss of muscle viability, and necrosis [3,4], which may affect surgical outcome [5,6]. In severe cases, lower extremity IRI is accompanied by distant organ

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damage, affecting lung, liver, kidney, or intestine, and may lead to the development of the multiple organ dysfunction syndrome [7].

Reports indicate that patients undergoing major vascular surgery with global and regional ischemia demonstrate shedding of the glycocalyx [8]. Under normal conditions, this negatively charged network of proteoglycans, glycosaminoglycans, and plasma proteins, coats the vascular endothelium and is responsible for its anti-inflammatory and anticoagulatory properties [9]. Consequently, glycocalyx shedding during ischemia and reperfusion changes the anti-inflammatory and anticoagulant endothelial surface into a proinflammatory and procoagulant surface and promotes vascular permeability [8,10]. Events that arise because of reperfusion, which significantly contribute to IRI, are the activation of the complement, coagulation, kinin, and fibrinolytic systems [11]. The complement system can be activated *via* three different pathways, which converge in the formation of the membrane attack complex, leading to lysis of the affected cell [12]. In 2006, Zhang *et al.* [13] identified non-muscle myosin heavy chain type II as a neoepitope for natural antibodies. Because of the hypoxic state during ischemia, non-muscle myosin heavy chain type II is mobilized to the cell surface and can be recognized by natural antibodies resulting in complement activation [14]. The coagulation system can be initiated *via* the intrinsic pathway, through factor XII by contact activation, or *via* the extrinsic pathway, where tissue factor–factor VIIa complexes are formed. These may lead to intravascular clotting, vessel occlusion, or thrombotic pathology [15,16]. Edema formation can be induced by the kinin system. Activation and the subsequent binding of bradykinin and des-Arg9-bradykinin to bradykinin receptors lead to vasodilation and increased vascular permeability [17]. Bradykinin is formed either by contact activation, in which FXIIa converts prekallikrein (pre-KK) to kallikrein (KK), or *via* the tissue pathway [18], where conversion is an enzymatic intracellular process, requiring a cell-derived cofactor or protease that activates pre-KK. However, other inflammatory mediators, such as leukotriene B4 or histamine, have also been shown to contribute to edema formation [19,20].

The synthetic polyanion low-molecular-weight dextran sulfate (DXS) is known for its heparin-like anticoagulant and complement inhibitory activity and acts by potentiating C1 esterase inhibitor-mediated inactivation of C1s and binding of factor H from plasma [21–23]. DXS modulates the biological effects of contact activation by inhibiting intrinsic coagulation without affecting the fibrinolytic potential of FXIIa and KK [24]. It was shown that DXS can act like a “repair coat” by functionally replacing the shed glycocalyx, thereby protecting the endothelium from damage [25]. *In vivo* work in our laboratory demonstrated that DXS significantly protects from IRI in a closed chest porcine model of acute myocardial infarction [26]. Furthermore, we showed that DXS attenuates IR-induced acute graft injury and facilitates long-term survival in a rat model of heart transplantation [27].

The aim of the present study was to investigate the effect of DXS on peripheral muscle IRI by using a rat hind limb model and analyze the underlying mechanisms. We hypothesized that the complement and coagulation systems are primarily responsible for skeletal muscle IRI in the presented rat model and the DXS treatment would maintain muscle viability and reduce local edema formation as well as distant lung damage.

2. Materials and methods

2.1. Animals and housing

All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and the Swiss animal protection law, and were approved by the Animal Experimentation Committee of the Cantonal Veterinary Service (Canton of Bern, Switzerland). Before experimentation, male Wistar rats weighing between 250 and 350 g (wild type, bred at the central animal facility, University of Bern, Switzerland) were kept in groups of three in clear 1500 cm^2 Euro-standard Type IV S cages (Tecniplast, Buguggiate, Italy) under standard housing conditions with food and water *ad libitum*. After surgical intervention, animals that were subjected to 24 h of reperfusion were kept separated. Cages were individually ventilated at 20 ± 2°C and 45%–65% relative humidity.

2.2. Reagents

Low-molecular-weight DXS (MW 5000), dissolved at 20 mg/mL in citric acid–buffered NaCl 0.9%, pH 5.9, was provided by TikoMed AB (Viken, Sweden).

2.3. Experimental groups

Rats were divided into five groups. On the basis of the preliminary experiments in our laboratory, experimental groups of 2 h ($n = 8$) and 24 h of reperfusion ($n = 6$) received 20 mg/kg of DXS 10 min before blood flow restoration (DXS groups) [26,27]. Control groups subjected to either 2 h ($n = 7$) or 24 h ($n = 6$) of reperfusion received no DXS (non-treated). Tissue samples from normal, healthy rats undergoing no surgical intervention ($n = 4$) were included for comparison. The different n numbers in the DXS and non-treated groups are explained by the exclusion of rats because of surgical complications. The 2- and 24-h reperfusion periods were selected because edema formation and complement activation are already detectable after 2 h. Furthermore, during 2 h of reperfusion rats stay under anesthesia, which reduces potential pain, distress, and discomfort. The 24-h time point was chosen to see whether the degree of injury worsens and investigate any delayed effects of DXS.

2.4. Anesthesia and analgesia

A total of 2.5% isoflurane in oxygen was used for anesthesia induction in a special box and later maintained by inhalation of 1.5% isoflurane using a nose mask. Analgesia was provided by subcutaneous injection of 0.05 mg/kg of buprenorphine (Temgesic, Reckitt Benckiser, Switzerland AG) 30 min before surgical intervention. The total duration of anesthesia was approximately 6h. For 2h reperfusion the rats were kept under anesthesia until euthanasia. For the 24-h reperfusion period the rats were allowed to wake up and buprenorphine injection