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Impact of gradual blood flow increase on ischaemia–reperfusion injury in the rat cremaster microcirculation model

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Summary *Introduction:* We aimed to evaluate the impact of gradual blood reperfusion on ischaemia–reperfusion injury and to explain the pathophysiology of reperfusion injury in a rat cremaster muscle microcirculation model.
Materials and Methods: Twenty-four Sprague–Dawley rats weighing 150–200 g were evaluated in three groups. Cremaster muscles were prepared for microcirculatory observations. Group I ($n = 8$, control): no ischemia was induced. Group II ($n = 8$, acute reperfusion): microclamps were applied to the right external iliac vessels for 150 min, then venous and arterial clamps were released at once. Group III ($n = 8$, gradual reperfusion): microclamps were applied to the right external iliac vessels for 150 min, and then the first venous clamp was released; the arterial clamp was opened gradually by a specially designed microclamp holder (Sheey ossicle holding clamp). In all groups, following a wait of 150 min blood flow velocity was measured for 15 min and then the animals were reperfused freely for 1 h. Next, red blood cell velocity, vessel diameters, functional capillary perfusion and endothelial oedema index were analysed, and rolling, migrating and adhesing leukocytes and lymphocytes were counted. All observations were videotaped for slow-motion replay. Muscle damage was evaluated histologically.
Results: In the acute clamp release group, blood velocities increased up to 600% of their pre-ischaemic values during the post-ischaemia–reperfusion period. The numbers of rolling, adhering and transmigrating leukocytes were significantly higher and histological evaluation revealed more tissue damage in the acute reperfusion group.

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Conclusion: Depending on histological and microcirculatory findings, gradual reperfusion was confirmed to reduce the intensity of reperfusion injury.

Re-establishment of blood flow is essential to salvage ischaemic tissues; on the other hand, reperfusion may lead to a process that paradoxically causes further damage to the ischaemic tissue, known as ischaemia—reperfusion (I-R) injury. With increasing duration of ischaemia the injury becomes more prominent and even irreversible. I-R injury may affect various organs and tissues including heart, kidney, lung, gut, brain and skeletal muscle. Moreover I-R injury may extend beyond the ischaemic area and damage the remote non-ischaemic organs [1]

For limiting or prevention of I-R injury, considerable effort has been made in developing new therapeutic strategies by the biotechnological and pharmaceutical industries. Many studies have been performed and various pharmacological and immunological agents have been used for this purpose.[2–6] Numerous therapeutic approaches have been proven to be effective in controlled experimental models; however, no single strategy has yielded a significantly superior result in clinical practice. Prevention of super oxygen radical occurrence and blockage of neutrophil migration and adhesion is the main purpose of these strategies.

Some surgical approaches have also been studied to limit or prevent I-R injury, including ischaemic preconditioning and gradual reperfusion. There are a limited number of studies focusing on the preventive effect of the gradual reperfusion technique and most of them were performed on myocardial muscle.[7–14] Gradual reperfusion was shown to prevent super oxygen radical occurrence and to inhibit neutrophilic action on the skeletal muscle I-R model.[15,16] However, there is some confusing information in the literature concerning the effect of gradual reperfusion on neutrophil function. Sato et al. stated that gradual reperfusion might cause increased neutrophil infiltration to the reperfused tissue; on the other hand, Atabay et al. and Unal et al. found decreased neutrophilic infiltration.[9,15,16]

The long-term objectives of this study were to characterize the impact of gradual blood reperfusion and to explain the pathophysiology of reperfusion injury upon the microcirculatory response. A rat testis cremaster muscle microcirculation model was used.

Materials and method

The present study was approved by the Animal Research Committee of The Cleveland Clinic Foundation. The animals used in this study received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals*, published by the National Institutes of Health. The Cleveland Clinic Foundation's animal care facility is accredited by the American Association for the Accreditation of Laboratory Animal Care. The animals were caged individually at room temperature, with a 12-h day/night cycle and free access to water and food. After microcirculatory measurements were completed, animals were killed with an intravenous injection of pentobarbital (100 mg/kg).

Vascular anatomy of the cremaster muscle

Proximal to the inguinal ligament the pudic—epigastric artery (PEA) arises directly from the iliac artery and runs through the abdominal wall muscle; after passing close to the proximal border of the inguinal ligament, it ends at the medial border of the rectus abdominis muscle, near the base of the penis. Then it gives one or two branches to the cremaster muscle [external spermatic artery(ies) — A1 arteriole(s)], penis, rectus abdominis muscle and perineum. The external spermatic artery (A1) runs through the cremaster muscle on its dorsal aspect. The pudic—epigastric vein (PEV) contributes to the PEA and joins the iliac vein.

Experimental model

The rat cremaster muscle flap model was used for measuring red blood cell (RBC) velocity, vessel diameters, functional capillary perfusion, leukocyte activation and endothelial oedema index.[17] Twenty-four Spraque—Dawley rats, weighing 150—200 g were divided into three groups containing eight animals each. In rats of this size the cremaster muscle is thin (200—300μ) and has minimal connective tissue, and thus with transillumination microcirculation studies can be easily performed.[18]

Group I ($n = 8$, control group): No ischemia was induced. Following a wait of 150 min, blood flow was measured using a Nikon fluorescence microscope equipped with Sony CCD-Iris camera and Javelin MDS solid state camera connected to an optical Doppler velocimeter for 15 min with 30-s intervals.

Group II ($n = 8$, acute reperfusion group): Acland microclamps were applied to the right external iliac artery and vein for 150 min. Following 150 min of ischaemia, first venous and then arterial clamp was released at once and blood flow rate was measured for 15 min during reperfusion by a Nikon fluorescence microscope equipped with Sony CCD-Iris camera and Javelin MDS solid state camera connected to an optical Doppler velocimeter.

Group III ($n = 8$, gradual reperfusion group): Acland micro-clamps were applied to the right external iliac artery and vein for 150 min. Following 150 min of ischaemia the venous clamp was released at once. The arterial clamp was opened gradually by a specially designed micro-clamp holder (modified Sheey ossicle holding clamp, Storz, N1705150, Karl Storz GmbH & Co., Tutlingen, Germany), to obtain a controlled blood flow rate. At the 30th s of arterial clamp release an arterial blood flow of 1/4 of preischaemic values, at the 60th s an arterial blood flow of 2/4 of preischaemic values, at the 90th s an arterial blood flow of 3/4 of preischemic values were obtained. At the 120th s blood flow was allowed to return to preischaemic values. For the following 13 min, arterial blood flow was controlled to be not over 1.5 times the preischaemic value.