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Prolonged Superficial Local Cryotherapy Attenuates Microcirculatory Impairment, Regional Inflammation, and Muscle Necrosis After Closed Soft Tissue Injury in Rats

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Background: Closed soft tissue injury induces progressive microvascular dysfunction and regional inflammation. The authors tested the hypothesis that adverse trauma-induced effects can be reduced by local cooling. While superficial cooling reduces swelling, pain, and cellular oxygen demand, the effects of cryotherapy on posttraumatic microcirculation are incompletely understood.

Study Design: Controlled laboratory study.

Methods: After a standardized closed soft tissue injury to the left tibial compartment, male rats were randomly subjected to percutaneous perfusion for 6 hours with 0.9% NaCL (controls; room temperature) or cold NaCL (cryotherapy; 8°C) (n = 7 per group). Uninjured rats served as shams (n = 7). Microcirculatory changes and leukocyte adherence were determined by intravital microscopy. Intramuscular pressure was measured, and invasion of granulocytes and macrophages was assessed by immunohistochemistry. Edema and tissue damage was quantified by gravimetry and decreased desmin staining.

Results: Closed soft tissue injury significantly decreased functional capillary density (240 ± 12 cm^{-1}); increased microvascular permeability (0.75 ± 0.03), endothelial leukocyte adherence ($995 \pm 77/cm^2$), granulocyte ($182.0 \pm 25.5/mm^2$) and macrophage infiltration, edema formation, and myonecrosis (ratio: 2.95 ± 0.45) within the left extensor digitorum longus muscle. Cryotherapy for 6 hours significantly restored diminished functional capillary density (393 ± 35), markedly decreased elevated intramuscular pressure, reduced the number of adhering ($462 \pm 188/cm^2$) and invading granulocytes (119 ± 28), and attenuated tissue damage (ratio: 1.7 ± 0.17).

Conclusion: The hypothesis that prolonged cooling reduces posttraumatic microvascular dysfunction, inflammation, and structural impairment was confirmed.

Clinical Relevance: These results may have therapeutic implications as cryotherapy after closed soft tissue injury is a valuable therapeutic approach to improve nutritive perfusion and attenuate leukocyte-mediated tissue destruction. The risk for evolving compartment syndrome may be reduced, thereby preventing further irreversible aggravation.

Keywords: closed soft tissue injury; cryotherapy; skeletal muscle; rats; microcirculation; leukocyte-endothelial cell interaction; intravital fluorescence microscopy

The overwhelming majority of injuries caused by recreational and competitive sport activities as well as high-velocity accidents typically involve moderate to severe trauma to the musculoskeletal soft tissues. Clinical and investigational experience has shown that primary traumatic muscle injury induces a plethora of pathological alterations known to converge in secondary structural and functional deterioration.[15,22,26,36,40,46] This secondary lesion growth is related to

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progressive microcirculatory impairment characterized by endothelial damage, local activation of the coagulation cascade, and marked leukocyte recruitment leading to, among others, decreased nutritive blood flow, reduced oxygen delivery, sustained cellular metabolism, and production of autodestructive free oxygen radicals.[9,17,18,40,52]

While the primary insult cannot be influenced therapeutically, the secondary lesion growth after closed soft tissue injury (CSTI) is amenable to certain interventions, including temporary immobilization, administration of analgesic/anti-inflammatory drugs,[4,22] and local cooling.[8,25] In principal, cryotherapy promotes structural and functional restoration and attenuates pain, which, in turn, ameliorates rehabilitation.[8] Local cooling is used as an integrated adjuvant treatment in the initial clinical management of musculoskeletal injuries, such as sprains, strains, lacerations, contusions of skeletal muscle, posttraumatic inflammatory disorders, and fractures. According to clinical and experimental studies,[21,45] decreasing tissue temperature reduces oxygen demand and cellular metabolic activity[12,13] and attenuates liberation of vasodilating mediators, thereby decreasing the microcirculatory burden by diminishing the circulating blood volume. This, in turn, attenuates the hydrostatic pressure known to promote local edema formation in the presence of damaged endothelial cells. In addition, cooling is thought to attenuate sustained leukocyte endothelial cell interaction and thrombus formation, and thus reduces the posttraumatically impaired tissue perfusion.

Previous experimental studies have shown that maximum posttraumatic microcirculatory disturbances and secondary tissue damage evolve over time and are not observed before 24 hours after injury.[29,40-42] Under clinical conditions, cryotherapy is usually initiated early after injury and maintained for at least several hours.[8,25] However, the detailed microcirculatory pathways underlying beneficial effects of local cooling on trauma-induced derangements in nutritive perfusion and leukocyte behavior in skeletal muscle remain to be established. Therefore, the aims of this experimental study are to assess the effects of prolonged local percutaneous cooling on changes in skeletal muscle microcirculation, leukocyte-endothelial cell interaction, inflammatory response, edema formation, and myonecrosis in rats subjected to traumatic CSTI and subsequent superficial cryotherapy for 6 hours.

MATERIALS AND METHODS

Experiments approved by the local animal right protection authorities were performed in accordance to the NIH guidelines for laboratory animal use.

Experimental Protocol

Male Sprague-Dawley rats (weight, 250-300 g) were randomly assigned to either a cooling group or a normothermic control group (n = 7/group). After CSTI, rats remained anesthetized for 6 hours during which either no or continuous percutaneous cryotherapy was maintained. Rats were then returned to their cages until further analysis at 24 hours after CSTI. After intravital fluorescence microscopy, muscles were removed for edema assessment and immunohistological analysis. Uninjured rats (n = 7) subjected to the same procedures served as shams.

Closed Soft Tissue Injury (CSTI)

Severe CSTI was induced in the antero-lateral compartment of the left midthigh in anesthetized and spontaneously breathing (isoflurane 1.5 vol.%, N_2O 0.5 L/min and O_2 0.3 L/min) rats. Closed soft tissue injury was performed with the PC-assisted high-pressure controlled impact injury (CII)-technique. For this, both hindlegs were shaved without exposing fascia or underlying muscle. In brief, a pneumatically driven bolt (11 mm diameter) with a flat tip was accelerated to an impact velocity of 7 m/s (~25 km/h), reversibly compressing the muscle by 11 mm at a contact time of 0.1 sec.[40] With these settings, a reproducible nonlethal focal injury (blunt trauma) to the left extensor digitorum longus (EDL) muscle was induced without concomitant bone fracture or incapacitating compartment syndrome.

Cryotherapy

Local tissue cooling was performed by continuously superfusing the shaved skin of the lower hindlimb with cooled saline (0.9% NaCL, 8°C). Surface temperature, maintained at 10°C, was monitored throughout the entire cooling period with a temperature needle probe (GTH 215 digital thermometer, Greisinger Electronic GmbH, Regenstauf, Germany) placed percutaneously beneath the fascia. Because of the further loss of cooling temperature in deeper tissue layers like fascias and subfascial skeletal muscle, the cooling temperature of 8°C (leading to a muscle surface cooling of 10°C) was used. Furthermore, pilot intravital microscopic studies have shown that decreasing tissue temperature below 6º to 8°C leads to cessation and breakdown of microvascular blood flow within capillaries and postcapillary venules, which makes any quantitative microcirculatory analysis of injured skeletal muscle by in vivo microscopy impossible. The rationale for choosing the cooling time of 6 hours was based on both the fact that shorter cooling times were partly already studied[2,9,21,44,45] and the current clinical practice for cryotherapy of musculoskeletal injury, which typically involves ice therapy of severe soft tissue damage for several hours.

Intramuscular Pressure (P_{im})

Intramuscular pressure was measured 24 hours after CSTI and in shams by percutaneously inserting a CODMAN microsensor (0.7 mm outside diameter; Johnson & Johnson Professional, Inc, Raynham, Mass) 8 mm vertically under the skin surface before surgically exposing the EDL muscle.[40]

Cannulation, Muscle Preparation, and Intravital Microscopy

Cannulation of the right jugular vein and the left carotid artery with polyethylene catheters (PE 50, 0.58 mm inner diameter; Portex, Hythe, Kent, UK) allowed the heart rate

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and blood pressure to be monitored and fluorescence markers to be injected. After the EDL muscle was surgically exposed, it was allowed to stabilize for 10 minutes before investigating skeletal muscle microcirculation using epi-illumination intravital multifluorescence microscopy with a modified high-resolution NIKON microscope (Optiphot, NIKON, Tokyo, Japan) equipped with a high-pressure mercury lamp (100 W) and a selective filter block system. This enabled fluorescence emission of fluorescein-isothiocyanate (FITC)-dextran (excitation/ emission wavelength: 450-490 nm/>580 nm), and rhodamine (530-560 nm/>580 nm) to be detected. Microvascular images and video sequences were recorded using a CCD-camera (FK 6990-IQ, Pieper, Schwerte, Germany) and transferred to an SVHS-video recorder (HR-S4700EG/E, JVC, Friedberg, Germany) for offline analysis, resulting in a 940-fold final magnification on the video screen. Contrast between erythrocytes and plasma was enhanced by injecting FITC-dextran intravenously (5%, 150 000 mol wt; 15 mg/kg body weight; Sigma Chemical, Deisenhofen, Germany) before each investigation. Injecting rhodamine 6G (0.1%, 0.15 mg/kg body weight; Sigma Chemical, Deisenhofen, Germany) enabled in vivo imaging of leukocytes.[47] Phototoxicity was limited by restricting continuous light exposure to 60 seconds.[35,39]

Microcirculatory Analysis

Quantification of posttraumatic skeletal muscle microcirculation and microvascular parameters was performed by the same investigator, who was blinded to the status of the rats. Videotaped sequences were evaluated by a frame-to-frame analysis using a computer-assisted image analysis program[23,51] for microvessel diameters, functional capillary density (FCD), microvascular permeability, and red blood cell velocity (V_{RBC}). Functional capillary density quantified by the length of erythrocyte-perfused capillaries per observation area (cm^{-1}) reflects nutritive tissue perfusion and oxygen delivery.[32] The microvascular permeability ratio depicting integrity of the endothelial barrier was assessed by computer-assisted densitometric quantification of fluorescence intensity in the perivascular and intravascular areas, that is, plasma gaps between erythrocytes in 4 fields per image. Centerline red blood cell velocity in capillaries and venules was measured from a line shift diagram by drawing a line along the length of the vessel in the center of the vessel and assessing the vertically aligned pixels under this line during a 10-second observation period.[23,51] Rolling and adherent leukocytes ("rollers" and "stickers") were counted for 30 seconds along a 100-µm vessel segment. Rollers were defined as slow passage of leukocytes moving along the vessel wall with a velocity less than 40% of centerline velocity and expressed in percent of total leukocyte flux.[3,28] Stickers were defined as leukocytes firmly attached to the endothelium of postcapillary venules for at least 20 seconds. Assuming cylindrical microvessel geometry, leukocyte adherence was expressed as nonmoving cells per endothelial surface (n/mm^2), calculated from the diameter and length of the microvessel segment analyzed. Leukocyte-endothelial cell interaction is a dynamic process that typically involves a sequence of events ranging from initial intermittent endothelial contact, continuous rolling with decreasing rolling velocity to total stop, and adherence with subsequent extravasation. Temporary leukocyte adherence (leukocyte rolling) as a precondition for permanent leukocyte adherence ("leukocyte sticking") and subsequent extravasation has been shown to be an essential trigger for the development of neutrophil-mediated tissue damage in many microcirculatory disorders ranging from ischemia-reperfusion, infection, transplant rejection, or shock.[48] Based on these observations, therapeutic approaches aimed at inhibition of leukocyte adherence and prevention of tissue destruction by neutrophils have been successfully developed. The major components of these leukocyte-mediated pathways are production of reactive O_2 radicals, release of cytotoxic enzymes, activation and expression of adhesion molecules, and increase in hydraulic flow pressure due to microvascular adherence. All these findings are converging on a self-perpetuated positive feedback cycle that promotes a persistent pro-inflammatory state with associated tissue damage and skeletal muscle dysfunction.[5,33,37]

Quantification of Edema Formation

At 24 hours after CSTI, edema formation was assessed gravimetrically by calculating the wet-to-dry-weight ratio of the left (traumatized) and contralateral (noninjured) EDL muscle (edema index: EI = left/contralateral EDL).[40]

Immunohistochemistry

Parafin processed 3-µm sections were used for avidin-biotin-complex and diaminobenzidine immunohistochemical staining for muscle specific class III intermediate filament desmin [monoclonal mouse antihuman desmin, clone D33 (1:50); DAKO, Glostrup, Denmark], rat neutrophilic granulocytes [mouse antirat granulocyte antibody, Clone: HIS48 (1:30); Pharmingen, San Diego, Calif, USA], and rat macrophages [ED-1 (1:200), DAKO, Glostrup, Denmark].

The extent of myonecrosis was assessed by planimetric analysis of areas void of desmin staining (indicator of early phase of muscle damage)[24,34,50] compared with areas with positive desmin immunostaining and calculation of the desmin ratio (immunonegative/immunoreactive area). Leukocytes and macrophages were analyzed by counting HIS48- and ED-1 positive cells in at least 15 randomly selected fields of 60 000 μm^2 per animal and expressed as total number of immunoreactive cells per square millimeter of tissue surface. Quantitative analysis was performed using a computer-assisted interactive image analysis system (Quantimed Image analysis, LEICA Instruments, Cambridge, UK).

Statistical Analysis

Results are presented as mean ± SEM and 95% confidence intervals (CI). Differences between groups were tested by 1-way analysis of variance (ANOVA) followed by post-hoc Bonferroni-correction for multiple comparisons. To assess the correlation between skeletal muscle viability (desmin ratio) and leukocyte-endothelial cell interaction (number of permanently endothelial-adhering leukocytes) and intramuscular neutrophilic granulocyte accumulation (number of HIS48

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immunoreactive cells), linear regression analysis was used. Differences were rated significant at $P < .05$. Statistical analysis was performed with Sigma Stat 2.0 (Jandel Scientific, San Rafael, Calif).

RESULTS

Macrohemodynamics

Heart rate (HR) and mean arterial blood pressure (MABP) remained unchanged and within normal limits at all time points. There were no differences between the groups (cryotherapy: 95 ± 2 mmHg, 95% CI: 10.7; no cryotherapy: 108 ± 5 mmHg, 95% CI: 5.3; sham: 117 ± 2 mmHg, 95% CI: 5.4). Anaphylactoid reactions to FITC-dextran were not observed.

Intramuscular Pressure

At 24 hours after CSTI, P_{im} was significantly increased compared with uninjured rats and markedly decreased by prolonged local cryotherapy (Figure 1).

Skeletal Muscle Microcirculation

At 24 hours after CSTI, FCD was significantly decreased (Figures 2 A, D, and G) in the normothermic rats (Figure 3A). Concomitantly, microvascular permeability ratio was markedly increased (0.75 ± 0.1 vs 0.55 ± 0.05; $P < .05$), and venular diameters were significantly widened compared with nontraumatized rats (Figures 2 B, E, H, and 3B).

Cryotherapy restored FCD (Figure 3A) and significantly decreased venular diameter to control values. Velocity within the venules was signficantly increased, exceeding control levels approximately 2-fold (Figure 3B). The microvascular permeability, diameter, and velocity within the capillaries remained fairly unchanged (Table 1).

Local Inflammatory Response

At 24 hours after CSTI, adherent leukocytes (Figures 2 C, F, I, and 4A) as well as neutrophilic granulocytes and macrophages within the EDL muscle were significantly increased compared with noninjured shams (Figure 4B).

Prolonged cryotherapy significantly reduced the number of adhering leukocytes (Figures 2 C, F, I, and 4A) without influencing the increased number of rolling leukocytes [38% ± 8% (cryotherapy) vs 34% ± 2% (no cryotherapy) vs 22% ± 4% (shams)]. Local cooling moderately decreased granulocytes and significantly reduced macrophages (Figure 4B).

Edema Formation

By 24 hours, CSTI significantly increased edema formation within the EDL muscle [1.13 ± 0.03 (no cryotherapy) vs 1.01 ± 0.03 (shams)] ($P < .05$). Local superficial cryotherapy maintained during the initial 6 hours after CSTI did not *significantly* reduce edema formation (1.11 ± 0.02).

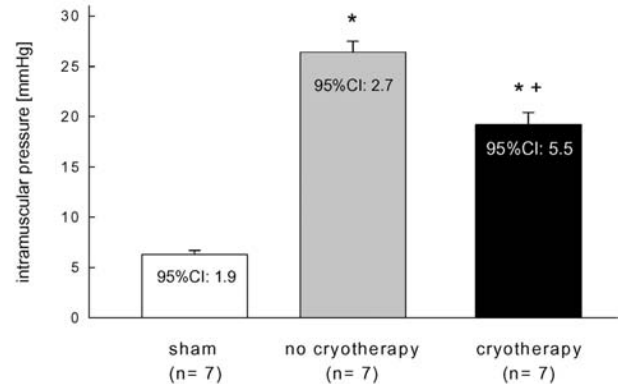

Figure 1. Local cryotherapy significantly reduced intramuscular pressure (P_{im}) compared with control rats. ($^{*}P < .05$ vs shams; $^{+}P < .05$ vs no cryotherapy).

Tissue Damage and Inflammatory Cell Response

Closed soft tissue injury significantly increased desmin ratio (immunonegative/immunopositive area) as determined by planimetric analysis reflecting decreased desmin-immunoreactivity at 24 hours compared with shams. Prolonged cryotherapy was associated with decreased desmin ratio, that is, preservation of desmin immunopositivity (Figures 5 and 6). Regression analysis of desmin ratio, reflecting loss of desmin immunoreactivity (early myonecrosis) and both endothelial leukocyte adherence and intramuscular immunoreactivity for neutrophilic granulocytes revealed a positive correlation (Figure 7) between sham-operated, cooling, and normothermic groups ($R = 0.58$ and $R = 0.85$).

DISCUSSION

Experimental contusion to the rat extensor digitorum longus (EDL) muscle induced significant structural and functional alterations reflected by microcirculatory dysfunction, activated local inflammation, increased intramuscular pressure, edema formation, and muscle fiber damage. These changes were attenuated, in part, by prolonged superficial local cryotherapy initiated immediately after injury and maintained for 6 hours. Thus, the initial hypothesis of cooling-induced reduction of posttraumatic microvascular dysfunction, inflammatory cell reaction, structural impairment, and improved tissue survival was confirmed.

CSTI and Microcirculatory Impairment, Inflammatory Response, and Tissue Damage

The evolving tissue damage incorporating primarily uninjured tissue is related to trauma- and ischemia-induced alterations. Sustained plugging of microcirculation decreasing FCD is related to uncontrolled thrombus formation, which aggravates the already impaired muscle perfusion. Inadequate perfusion and oxygen delivery with ensuing

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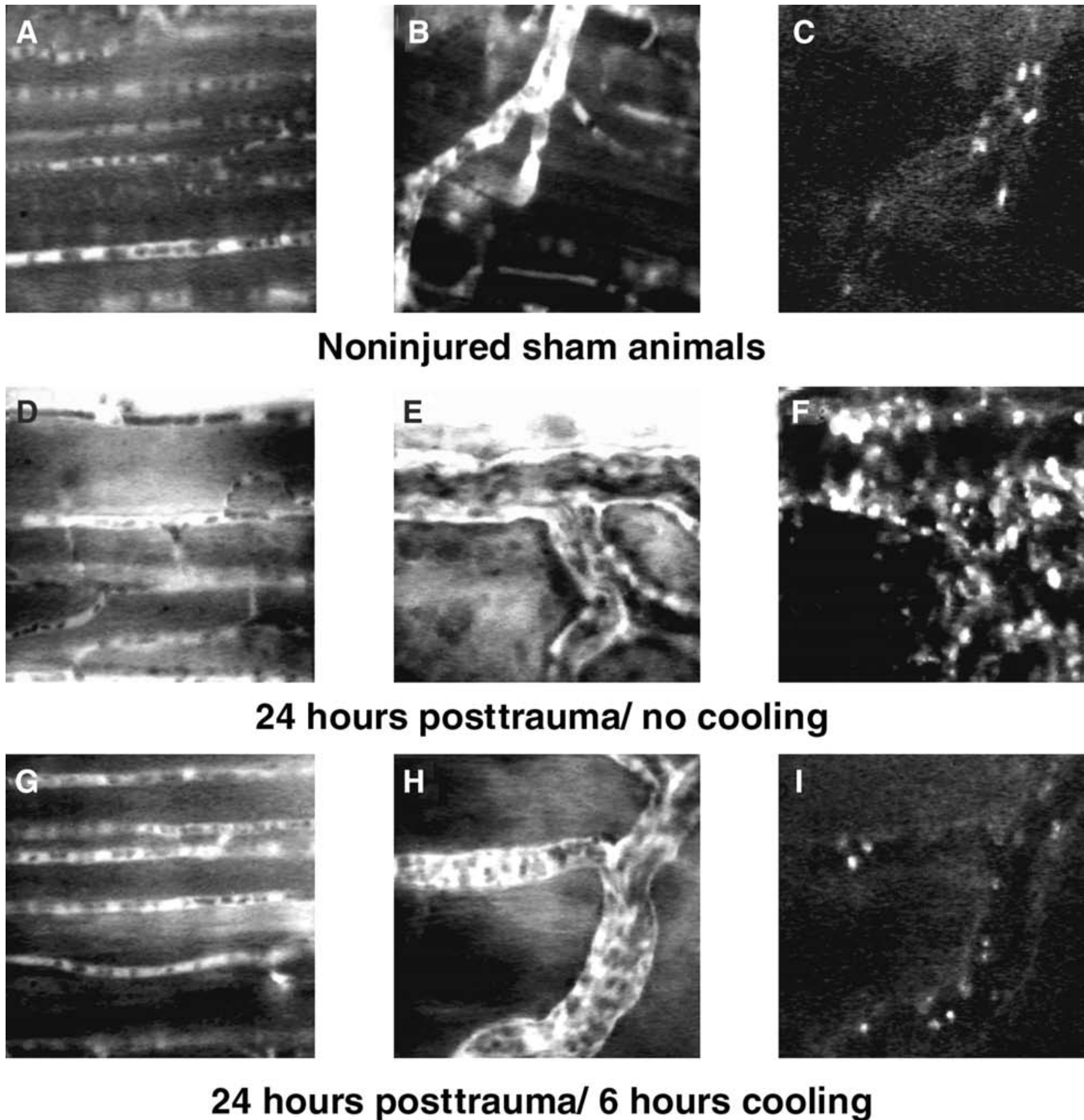

Figure 2. Intravital fluorescence microscopic images of microvascular perfusion in capillaries and postcapillary venules, and leukocyte-endothelial cell interaction in rat skeletal EDL-muscle. In noninjured animals, homogeneous capillary perfusion was found (A) and postcapillary venules (B) showed only a few leukocytes adhering to the microvascular endothelium of the identical venular segment (C). Closed soft tissue trauma resulted in a marked decrease in functional capillary density (D) and increase in microvascular leakage (E) as well as leukocyte adhesion (F) at 24 hours after injury. Continuous superficial local cryotherapy for the first 6 hours after trauma significantly impaired capillary dysfunction (G), partly restored endothelial integrity (H), and decreased venular accumulation and adherence of leukocytes (I).

hypoxia/ischemia results in cellular damage accompanied by sustained intracellular water accumulation. Uncontrolled leakage of plasma and blood across the damaged endothelium reflected by sustained leakage of FITC-dextran results in extracellular water accumulation. These changes, in turn, increase muscle swelling, intercapillary distance, and intramuscular pressure, thus compressing skeletal microcirculation and further impairing cellular viability within the

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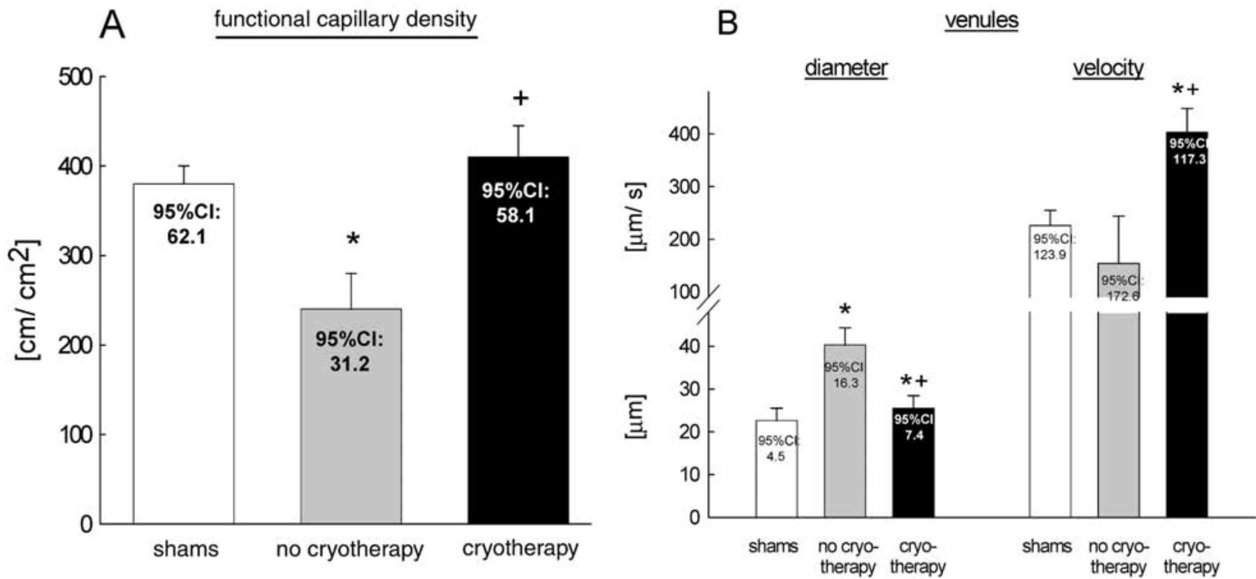

Figure 3. CSTI-induced significant microcirculatory changes were, in part, reversed by local cryotherapy, as reflected by restored functional capillary density (A), vessel narrowing, and increased velocity in venules (B). (*$P < .05$ vs shams; +$P < .05$ vs no cryotherapy).

TABLE 1
Microvascular Diameter, Permeability, and Velocity[a]

Group	Diameter (µm)	Microvascular Permeability	Red Blood Cell Velocity (µm/s)
Sham	5.1 ± 0.4 (1.06)[b]	0.72 ± 0.05 (0.13)	232 ± 31 (83.5)
Cryotherapy	5.7 ± 0.3 (0.74)	0.70 ± 0.03 (0.08)	212 ± 11 (28.8)
No cryotherapy	5.7 ± 0.1 (0.28)	0.74 ± 0.03 (0.07)	170 ± 14 (34.1)

[a]Microvascular diameter, microvascular permeability, and red blood cell velocity in capillaries of extensor digitorum longus (EDL) muscle did not significantly change in response to closed soft tissue trauma with or without prolonged superficial local cooling when compared with sham-operated controls.
[b]Values were expressed as mean ± SEM (95% CI).

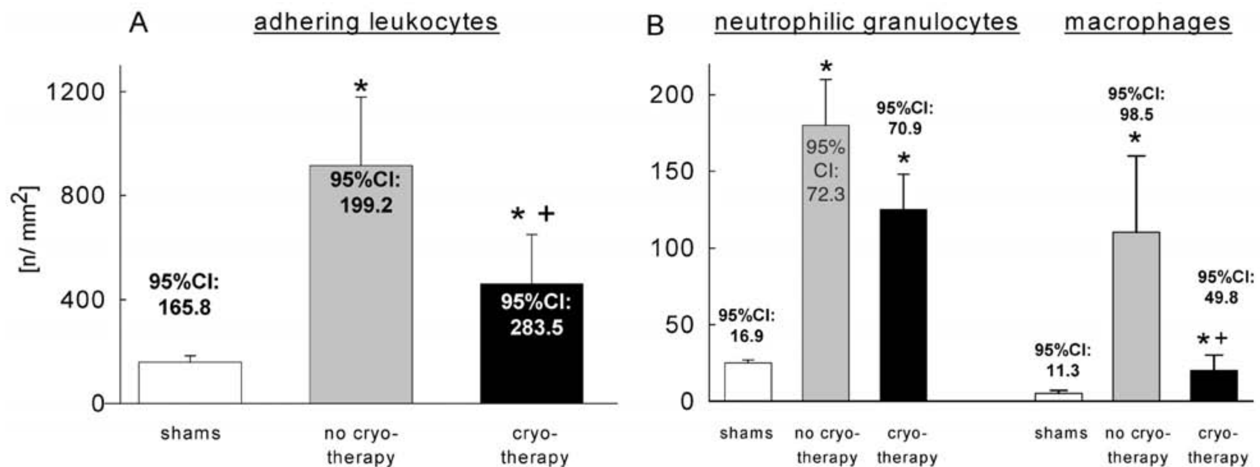

Figure 4. CSTI-induced local inflammatory response was partially reversed by local cryotherapy, reflected by significantly reduced "stickers" (A) and macrophages (B) within the injured EDL muscle (*$P < .05$ vs shams; +$P < .05$ vs no cryotherapy).

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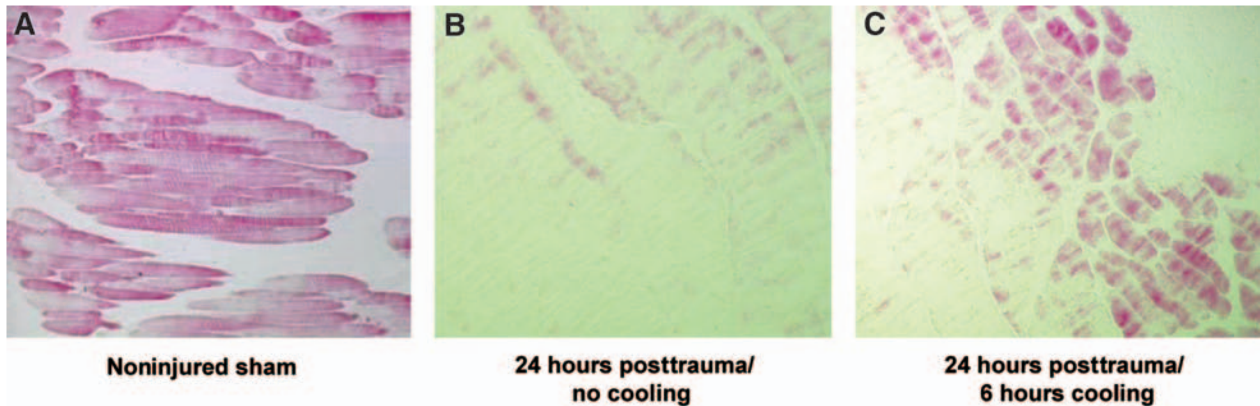

Figure 5. Immunodetection of desmin at 24 hours after trauma. EDL muscle of (A) noninjured animals (sham) displays homogeneous local desmin expression. Decreased immunostaining for desmin at 24 hours after trauma (B) demonstrates reduced skeletal muscle viability. Local cooling (C) resulted in partial reversal of trauma-induced decrease in desmin expression.

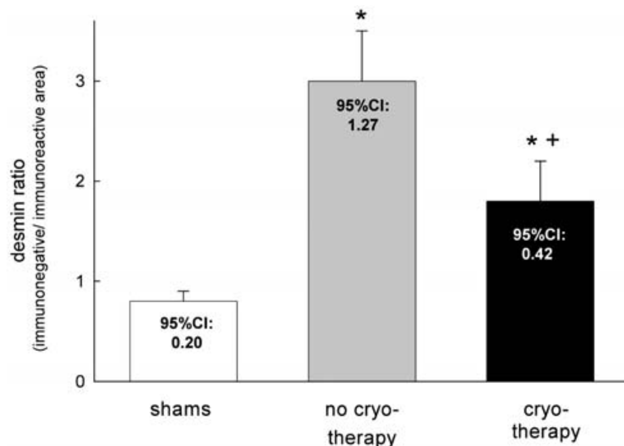

Figure 6. At 24 hours after CSTI, desmin ratio (planimetric analysis of immunonegative/immunoreactive area) reflecting skeletal muscle viability was significantly increased compared with uninjured rats. Local cryotherapy significantly reduced the extent of structural muscle damage. (*$P < .05$ vs shams, +$P < .05$ vs no cryotherapy).

muscular compartment. After initial early vasoconstriction following muscle injury, sustained upregulation of endothelial nitric oxide synthase (eNOS) and inducible NOS (iNOS) activity resulting in liberation of the potent vasodilator nitric oxide (NO) increases local tissue perfusion. This hyperperfusion, assessed by laser Doppler flowmetry beginning at 6 hours, lasts up to 4 days after experimental muscle crush injury.[38] Nitric oxide-induced vasodilation appears to mediate the vasodilation reflected by an increased venular diameter at 24 hours after CSTI as determined by intravital microscopy.

Activation of intracellular second and third messenger cascades upregulates translational and transcriptional activities, thereby inducing liberation of pro-inflammatory cytokines and rapid expression of ICAM-1 and P-selectin. These alterations, in turn, mediate cross linking of other leukocyte surface receptors.[6] In agreement with previous studies,[10,30,40,52] we found that CSTI significantly increased the number of rolling and sticking leukocytes. While posttraumatic loose adherence of leukocytes to endothelial cells is mediated by selectins,[14,27] firm attachment is based on stimulated β_1-/β_2-integrin dependent adhesion to endothelial cell membranes containing ICAM-1.[43] Sustained adherence of leukocytes supported by released cytokines, for example, TNF-α, facilitates inflammatory penetration and invasion of the injured tissue where these leukocytes perpetuate ongoing destructive processes before removing cellular debris. Release of pro-inflammatory cytokines and free oxygen radicals, in turn, aggravate underlying tissue injury and contribute to edema formation and cell damage. Further evidence for this leukocyte-mediated tissue destruction is provided by the observed positive correlation of loss in desmin immunoreactivity and both the endothelial leukocyte adherence and the intramuscular accumulation of HIS48-immunoreactive neutrophilic granulocytes (Figure 7). Structural damage as reflected by the presently observed loss in desmin immunoreactivity (increased desmin ratio) can induce and perpetuate functional impairment, which prolongs anastasis and length of hospitalization.

Effects of Local Cryotherapy After CSTI

Local cryotherapy is protective after ischemia-reperfusion injury,[44] inflammation,[2,49] and trauma[10,42] by reducing microcirculatory dysfunction, endothelial leakage, thrombus formation, leukocyte activation, and progressive tissue damage. In this context, upregulating heme oxygenase and nitric oxide synthase,[2,11] decreasing liberation of pro-inflammatory cytokines, for example, TNF-α, and free oxygen radicals, reducing release of histamine and bradykinin by activated leukocytes and damaged endothelial cells as well as attenuating synthesis of thrombogenic agents, for example, thromboxanes,[1,7] and lowering oxygen consumption[12,13] are potential targets. In addition, the reduced P_{im} (compartment) may also be obtained by inhibiting the initial bleeding into the injured tissue. Decrease in initial bleeding may

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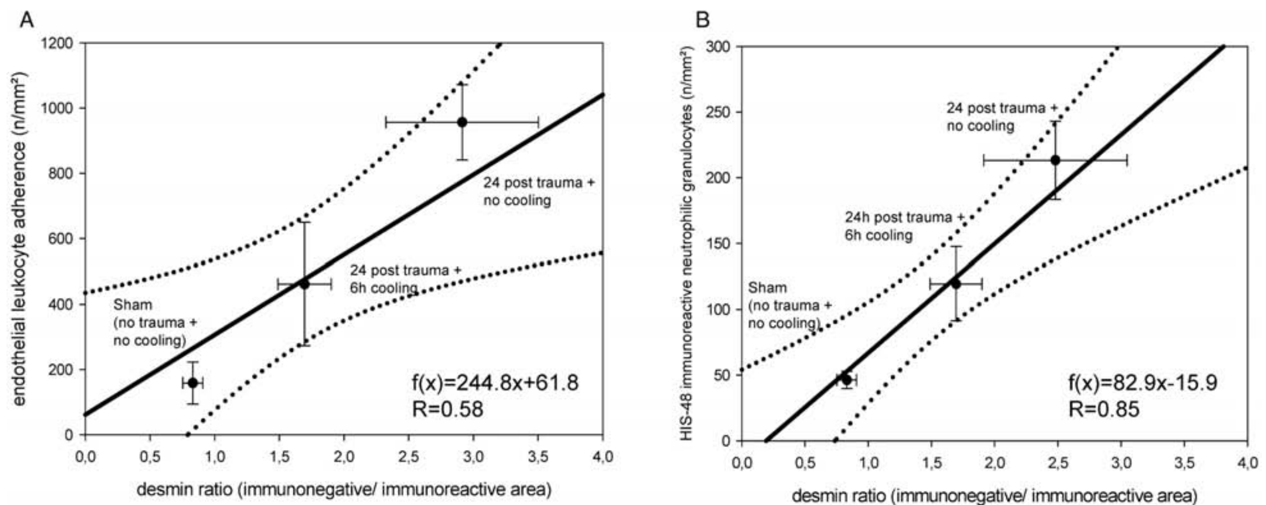

Figure 7. Regression analysis between desmin ratio (immunonegative/immunoreactive area) and values of (A) endothelial leukocyte adherence (number of permanently adherent leukocytes) and of (B) HIS48-immunoreactive neutrophilic granulocytes in rat EDL muscle. Values are given as mean ± SEM, obtained in sham-operated animals and at 24 hours after CSTI in normothermic controls (no cooling), and with cooling maintained for the first 6 hours. Dotted lines represent 95% CI. R, regression coefficient.

be caused by initial vasoconstriction and platelet aggregation, which are enhanced by cryotherapy. Furthermore, the surviving part of the muscle fiber is demarcated from the necrotic part by a specific structure called the contraction band, a condensation of cytoskeletal material.[20] Thus, cryotherapy may also result in a restricted spreading of the ischemia by the reduction of the hydrostatic pressure on viable muscle fibers.

Microcirculatory Impairment

Significantly increasing FCD reflecting nutritive perfusion[32] suggests improved tissue oxygenation essential to support and maintain cellular viability and prevent sustained energetic impairment known to compromise structural and functional cellular integrity. Ameliorated perfusion in conjunction with the attenuated inflammatory response appears to account for the significantly attenuated cell damage, myofiber necrosis, and muscle swelling. Preservation of endothelial integrity in terms of decreased FITC-dextran leakage into the extracellular space decreases the intramuscular pressure. The significant increase in V_{RBC} in face of a concomitant decrease in venular diameter is suggestive of sustained volumetric blood flow. This increase in postcapillary outflow may result in reduced hydrostatic capillary filtration pressure and sustained clearance of otherwise accumulating autodestructive mediators possibly related to an increased surface area for reabsorbtion as suggested by Smith and colleagues.[42]

Local Inflammatory Response

Sustained mediator-activated attraction, adherence, and chemotaxis of circulating leukocytes, in conjunction with degranulation and release of proteolytic enzymes and pronounced endothelial cellular membrane damage via increased release of oxygen-derived free radicals, contribute to the progressive structural and functional deterioration.[28]

The presently induced prolonged cryotherapy significantly reduced venular adherence of leukocytes and attenuated presence of immunocompetent cells (neutrophilic granulocytes, macrophages), which was accompanied by reduced structural damage (decreased loss of desmin immunoreactivity) within the damaged EDL muscle. The concept of reducing secondary leukocyte-perpetuated tissue damage[48] by local cryotherapy is further supported by the presently found positive correlation between increased skeletal muscle viability, that is, decreased desmin ratio, and reduced endothelial and parenchymal leukocyte activation following local cooling. Several experimental studies have shown that trauma-induced interactions between leukocytes and surface receptors can be inhibited by cooling.[6] In this context, hypothermia may block E-selectin transcription[16] and reversibly inhibit neutrophil adherence. In vitro studies have revealed that mechanical and adhesive properties of neutrophils are markedly altered by cooling as the cytoplasmic viscosity and structural rigidity of leukocytes are increased and leukocyte binding via the nonfunctional integrin CD11b/CD18 is reduced.[31] In a recently published study, cooling-associated protection against TNF-α–induced microcirculatory dysfunction and apoptotic cell death is mediated by metabolites of activated heme oxygenase and nitric oxide synthetase known to counterregulate vascular constriction early after injury.[2] Contrary to the neutrophil mediated tissue damage in skeletal muscle microcirculatory disorders following skeletal muscle trauma and ischemia, macrophages may have a differential role. As reported in studies by Hurme et al,[20] the phagocytosis of the necrotic material is a highly specific and selective process, as the preserved cylinders of the basal laminas surrounding the necrotized parts of the injured myofibers survive and are left intact by the macrophage attack. Consequently,

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these cylinders serve as scaffolds inside which the viable satellite cells begin the regeneration process.[19] Thus, it is also possible that cryotherapy-induced decrease in extravasation and activity of macrophage into the injury area may partially delay and indeed hinder the early regeneration of skeletal muscle slightly after the contusion injury.

CONCLUSION

The present experimental study provides in vivo evidence that cooling-induced reduction of secondary tissue damage is mediated by a decrease in posttraumatic capillary dysfunction and leukocyte-endothelial cell interaction. From a clinical perspective, prolonged local superficial cooling initiated early after traumatic skeletal muscle injury is a valuable therapeutic approach to improve nutritive tissue perfusion, preserve cellular viability, attenuate leukocyte-mediated tissue destruction, and to reduce the risk for evolving compartment syndrome, thereby preventing further irreversible aggravation.

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