Nitroglycerine Reduces Neutrophil Activation and Acute Damage in Latissimus Dorsi Muscle Grafts

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Background. **Damage to the latissimus dorsi muscle (LDM) may jeopardize a successful outcome to dynamic cardiomyoplasty. We and others have demonstrated muscle damage in LDM in various species including humans. Ischemia is now recognized to be an important contributory factor. We postulated that glyceryl trinitrate, a nitric oxide donor, might protect against ischemic endothelial dysfunction and so reduce resultant muscle damage.**

Methods. **In 20 adult rats the left LDM was mobilized on its thoracodorsal neurovascular pedicle and maintained as an orthotopic graft. Half of the animals received glycerol trinitrate intraoperatively and postoperatively for 24 hours. The other half served as untreated controls. Each group was further subdivided into two groups (n = 5 in each): animals in which the LDM was excised after 4 hours for myeloperoxidase studies, and animals in which the LDM was excised at 24 hours for analysis of muscle damage by histology and enzyme macrohistochemistry. Blood samples were taken at 24 hours for assay of plasma nitrite and nitrate as nitric oxide metabolites.**

Results. **Glycerol trinitrate-treated animals had higher plasma nitric oxide metabolite levels after 24 hours (after nitrate reductase treatment, total nitrite, 78.3 ± 11.8 nmol/mL, mean ± SEM) than controls (42.1 ± 3.7 nmol/mL, $p = 0.008$). The proportion of viable LDM in glycerol trinitrate-treated animals was greater than in untreated animals, mainly in the middle and distal regions of the graft (middle region, 96.3% ± 0.5% versus 75.7% ± 4.1%, $p < 0.001$; distal region, 94.4% ± 0.8% versus 40.9% ± 3.1%, $p < 0.001$). Macrohistochemical findings correlated well with the histologic findings. Myeloperoxidase activity (U/g) was markedly lower in glycerol trinitrate-treated LDMs, mainly in the distal part of the graft (glycerol trinitrate versus control, 20.5 ± 2.1 versus 40.9 ± 3.1 U/g, $p < 0.001$).**

Conclusions. **Glycerol trinitrate significantly reduced acute damage to the distal two-thirds of the mobilized LDM, possibly by modifying leukocyte activation and endothelial dysfunction associated with ischemic injury.**

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The latissimus dorsi muscle (LDM) can be mobilized as a functional graft to provide cardiac assistance in end-stage heart failure. In dynamic cardiomyoplasty, a pedicled LDM graft is transposed into the chest and wrapped around the cardiac ventricles. Electrical stimulation synchronized to the electrocardiogram results in contraction of the muscle wrap during ventricular systole.

A successful outcome of dynamic cardioplasty depends to an extent on the functional integrity of the LDM wrap. It is therefore a matter of concern that replacement of muscle by fibrous fatty tissue, mainly in the distal part of the LDM graft, has been observed both in animals [1] and in man [2, 3]. The origins of this damage are probably multifactorial, but there are strong indications that ischemia is a key contributory factor. This may be aggravated by the cumulative effects of badly chosen regimens of chronic electrical stimulation [4], but damage can also occur at an early stage, even in the perioperative and immediate postoperative period, as evidenced by large peaks of serum creatine phosphokinase [3].

The susceptibility of the LDM to ischemia after surgical mobilization is related to its blood supply. This comes from two sources: the thoracodorsal artery, which enters the proximal part of the muscle, and branches of the intercostal arteries, which perforate the chest wall and enter the distal part. The two vascular networks are interconnected through arterial anastomoses within the muscle [5, 6]. Anastomotic channels have been well documented by anatomic techniques, and recently we were able to confirm that they are functional under physiologic conditions of pressure and flow [7]. Thus, even after surgical mobilization and loss of the intercostal blood supply, the anastomotic channels could, in principle, enable the thoracodorsal artery to continue to perfuse the distal part of the LDM graft by means of an existing vascular network. However, the efficacy of these connecting vessels may be compromised at any early stage by a number of factors; these include electrocautery, handling and cooling of the LDM, and loss of normal resting tension during surgical mobilization. This would lead to a period of ischemia in the distal part of the

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muscle, with the possibility of subsequent ischemic and reperfusion injury.

There is considerable evidence for local endothelial regulation of vascular homeostasis by the production of nitric oxide to control tissue blood flow [8]. Ischemic injury to the endothelium interferes with this autoregulation and thus leads to inappropriate vasoconstriction, activation of leukocytes and platelets, and ultimately microvascular thrombosis and tissue damage [9]. We postulated that if ischemia were a significant factor in the early onset of muscle damage, then treatment with a nitric oxide donor might improve LDM viability. Studies focusing on ischemia-reperfusion injury in skeletal muscle have highlighted the important role of neutrophils in mediating acute damage. We have therefore measured myeloperoxidase (MPO) activity, which quantifies neutrophil accumulation, in the LDM shortly after surgical intervention.

Materials and Methods

Experimental Design

Animals were operated on and cared for in accordance with the Animal (Scientific Procedures) Act, 1986, which regulates animal experimentation in Great Britain and Northern Ireland. Twenty adult male Sprague-Dawley rats (Charles River, Kent, UK), weighing between 380 and 420 g, underwent surgical mobilization of the left LDM, with preservation of the thoracodorsal pedicle. Ten of the animals received glycerol trinitrate (GTN) therapy (group 1) and 10, which did not, served as controls (group 2). In each group, half of the animals were sacrificed after 4 hours and the right and left LDMs excised for MPO assays (groups 1a and 2a). The other 5 animals in each group were sacrificed after 24 hours, and LDMs were excised for assessment of muscle viability by enzyme macrohistochemistry and histology (groups 1b and 2b).

Glycerol Trinitrate Therapy

With the animal anesthetized and before any surgery was performed, a measured patch (18.3 mm^2) of GTN (Deponit, Schwarz Pharma Ltd, Chesham, UK), designed to provide sustained transdermal release of the drug at a rate of 10 mg/24 hours from a matrix-gel, was secured to a shaved area of interscapular skin of animals in group 1. An intravenous dose (25 μg/kg) of GTN (DBL, Warwick, UK) was administered into a femoral vein to provide systemic loading of the drug before any surgical intervention. In pilot experiments we established the systemic doses of GTN needed to cause a reduction of about 30% in the mean arterial pressure.

Anesthesia and Surgery

General anesthesia was induced and maintained with a 2:1 mixture of nitrous oxide and oxygen in combination with halothane (1% to 2%). Each rat breathed spontaneously throughout the procedure. A heating blanket, controlled by feedback from a rectal thermometer, regulated core body temperature within the normal range for rats (38° to 39.5°C). Analgesia was provided by intramuscular diclofenac sodium (Geigy Pharmaceuticals, Horsham, UK) given initially at a dose of 1 mg/kg and supplemented at 12-hour intervals when necessary. Blood gas analysis of samples (0.3 mL) taken from a femoral vein allowed intraoperative monitoring of respiratory function.

A left flank incision was made from the posterior axilla to the 11th rib to expose the underlying LDM. Blood vessels supplying the LDM other than the thoracodorsal artery were divided. The muscle was mobilized from its truncal attachments, raised as a pedicled graft, and replaced at its resting length with a running 5-0 Prolene (Ethicon, Somerville, NJ) suture to preserve the physiologic tension. Wounds were closed after hemostasis and the animal was allowed to recover. After 4 hours in groups 1a and 2a, and after 24 hours in groups 1b and 2b, animals were killed by exsanguination under anesthesia. Blood was collected in a heparinized tube for assay of plasma nitrites and nitrates. The experimental LDMs were excised at the same time as the undisturbed contralateral muscles, which acted as matched controls.

Plasma Nitrate Determination

Plasma samples were separated immediately from whole blood by centrifugation at 1,500*g* for 5 minutes and then stored frozen at −70°C pending analysis. Nitric oxide metabolites in plasma were measured as nitrites using the Griess reaction, after first converting nitrates to nitrite with nitrate reductase. Plasma (60 μL) was placed in a 1.5-mL Eppendorf tube with 10 μL nitrate reductase (5 U/mL, Sigma, Poole, UK) and 30 μL reduced nicotinamide-adenine dinucleotide phosphate (1.25 mg/mL, Sigma) and incubated at room temperature (19° to 23°C) for 30 minutes. Griess reagent (5% phosphoric acid, 1% sulfanilic acid, 0.1% *N*-(1-napthyl)ethylene diamine in deionized water; 200 μL) was added and the solution incubated at room temperature for a further 10 minutes. Protein was precipitated by addition of 10% trichloroacetic acid (200 μL), with thorough mixing, and microcentrifuged (MSE MicroCentaur, Sanyo Gallenkamp, Leighster, UK) at 13,400 g for 15 minutes at 4°C. Supernatant was transferred onto a microplate, and the absorbance was read at 540 nm on a microplate reader (Dynatech Laboratories MRX). Nitrite concentrations were determined from a standard curve for sodium nitrites (0.1 to 50 μmol/L) in pooled rat plasma. Values are expressed as nitrite in nanomoles per milliliter of plasma and have been corrected for interference in the spectrophotometric assay (by the use of internal standard sodium nitrites in experimental samples) and incomplete conversion of nitrate to nitrite by nitrate reductase (by the use of external standard sodium nitrate).

Myeloperoxidase Assay

Myeloperoxidase is a heme-containing enzyme found predominantly in neutrophils, and provides a suitable quantitative measure of neutrophil accumulation in skeletal muscle [10]. The assay has been established previously in our laboratory [11]. After excision, the LDM was