Oral vitamin C reduces the injury to skeletal muscle caused by compartment syndrome

S. R. Kearns,
A. F. Daly,
K. Sheehan,
P. Murray,
C. Kelly,
D. Bouchier-Hayes

From Beaumont Hospital, Dublin, Ireland

Compartment syndrome is a unique form of ischaemia of skeletal muscle which occurs despite patency of the large vessels. Decompression allows the influx of activated leucocytes which cause further injury. Vitamin C is a powerful antioxidant which concentrates preferentially in leucocytes and attenuates reperfusion-induced muscle injury. We have evaluated the use of pretreatment with oral vitamin C in the prevention of injury caused by compartment syndrome in a rat cremasteric muscle model.

Acute and delayed effects of pretreatment with vitamin C were assessed electrophysiologically. Vascular, cellular and tissue inflammation was assessed by staining of intercellular adhesion molecule-1 (ICAM-1) and by determination of the activity of myeloperoxidase (MPO) in neutrophils and tissue oedema.

Compartment syndrome impaired skeletal muscle function and increased the expression of ICAM-1, activity of MPO and muscle weight increased significantly. Pretreatment with vitamin C preserved muscle function and reduced the expression of ICAM-1, infiltration of the neutrophils and oedema.
either right orchidectomy only (control group), compartment syndrome only or vitamin C plus compartment syndrome (Table I). The animals treated with vitamin C received a dose of 2 g/kg daily (Roche Pharmaceuticals, Dublin, Eire) for five days before injury. Daily vitamin C was administered in 30 ml of drinking water and experiments were performed only in rats which had consumed the full five-day dose of vitamin C.

**Induction of compartment syndrome.** The preparation of the cremasteric muscle was performed in halothane-anaesthetised rats according to our previously described protocol. The isolated cremasteric neurovascular pedicle was introduced into the purpose-built compartment-syndrome chamber and the portal sealed with an angioplasty catheter (Fig. 1). The pressure in the angioplasty catheter was maintained at chamber pressure or below to prevent a tourniquet-like effect on the neurovascular pedicle. Elevation of chamber pressure to within 10 mmHg of the diastolic blood pressure for three hours was used to induce compartment syndrome. At the end of this period the pressure was released to simulate a fasciotomy. In the acute experiments, after reperfusion for one hour the cremasteric muscle was harvested. Muscle function, oedema, the expression of ICAM-1 and the activity of myeloperoxidase (MPO) were assessed. In the delayed groups the muscle was inspected for viability after decompression, returned to the abdominal cavity and the scrotum was sutured using Ethilon sutures (Johnson & Johnson, Brussels, Belgium). The rats were resuscitated and given intramuscular analgesia with buprenorphine (0.03 mg; Schering-Plough, Welwyn Garden City, UK). After 24 hours, they were re-anaesthetised and the cremasteric muscle was harvested for experimental testing. The rats were then killed using a lethal intracardiac dose of sodium pentobarbitone.

**Assessment of muscle function.** A strip of cremasteric muscle 2.5 x 0.5 cm in size from a central area was isolated. As previously described, the strip was maintained at 37°C in a bicarbonate buffer solution (Sigma Chemical Co Ltd, Irvine, UK) and the pH was corrected from 7.3 to 7.4. The pH and O₂ saturation were maintained by constant aeration with a 95%O₂/5%CO₂ gas mixture (BOC Gases, Dublin, Eire). The muscle was stimulated using supramaximal pulses (20 V, 2 msec square-wave duration and 40Hz) obtained from a pulse generator (Harvard stimulator; Harvard Apparatus, Edenbridge, UK). The isometric contraction of each muscle strip was assessed in response to a timed series of twitch and tetanic electrical stimuli. The muscle strip was then weighed (Oertling YA124 Analytical Balance; Avery Berkel, Warley, UK).

**Myeloperoxidase assay.** Measurement of MPO has been shown to be a reliable method of quantitatively assessing neutrophil sequestration. The activity of MPO was assayed spectrophotometrically (450 nm, Beckton-Dickinson, Mountain View, California) in weighed homogenised sections of cremasteric muscle as previously described. One unit of MPO was defined as that which degraded 1 µmol of H₂O₂ per minute at 25°C.

**Muscle oedema.** The wet-to-dry ratio is a simple assessment of tissue oedema. A separate section of freshly harvested cremasteric muscle was weighed (Oertling YA124 Analytical Balance; Avery Berkel), and then heated at 60°C...
in an oven (Gallenkamp Model IH-150; Sanyo Gallenkamp plc, Loughborough, UK) for 72 hours until such time that the weight had become constant. The difference between the wet and dry weight was recorded.

**ICAM-1 expression.** This was assessed in the acute groups using a standard immunocytochemical technique (StreptABComplex/HRP code K377; DAKO A/S, Glostrup, Denmark). A section of cremasteric muscle was mounted in an optimal cutting temperature compound (Tissue-Tek; Miles Laboratories, Elkhart, Indiana) and snap-frozen in liquid nitrogen. The positive control for the assays was taken as a delegated sample of cremasteric muscle seen to contain ICAM-1, this was used in each run. Negative controls were performed using irrelevant antisera and the unbound secondary antibody from the assay alone. Scoring was carried out by a blinded observer (KS) in a semiquantitative manner using the following grading system: 0, no stain; 1, weak stain, scant distribution; 2, weak staining, widespread distribution; 3, strong staining, scant distribution; and 4, strong staining, widespread distribution.21,22

**Statistical analysis.** The results were expressed as the mean or median with the 95% confidence interval (CI). Analysis of data was performed using the one-way analysis of variance for the comparison of multiple means (one-way ANOVA). Data were tested for normality and constant variance before analysis. Significant results were analysed using the post-hoc Tukey test. Data which were not normally distributed underwent log transformation before analysis and these were expressed as the median (95% CI). Significance was achieved if \( p < 0.05 \). Non-parametric data (ICAM-1) were analysed using the Kruskal-Wallis test followed by the Mann-Whitney U test on pairs of groups. Significance was achieved if \( p < 0.05 \).

**Results**

**Muscle function.** Twitch and tetanic muscle contractile function was expressed as the peak tension, in grams of ‘force’, achieved by each muscle. In the group with compartment syndrome muscle twitch contraction was acutely impaired (59.9 g, 95% CI 38.5 to 81.4) compared with the control group (202.4 g, 95% CI 154.5 to 250.3 g; one-way ANOVA, \( p = 0.001 \), \( p < 0.001 \) versus compartment syndrome alone; Fig. 2). Twenty-four hours after decompression muscle contraction had recovered (134.8 g, 95% CI, 95.5 to 174.1 g one-way ANOVA; \( p = 0.001 \); \( p < 0.01 \) versus compartment syndrome alone; Fig. 2).
twitch contraction in the compartment-syndrome group was still significantly impaired (50.4 g, 95% CI, 30.7 to 70.1) compared with the control group (108.5 g, 95% CI, 78.9 to 138.2; one-way ANOVA, p = 0.006; p < 0.01 versus compartment syndrome). In the group pretreated with vitamin C, fast fibre function was maintained at levels above those seen in the non-vitamin-C treated compartment-syndrome group (126.3 g, 95% CI, 91.2 to 161.4; one-way ANOVA, p = 0.006; p < 0.01 versus 24 hour compartment syndrome alone; Fig. 2a).

Tetanic muscle contraction was also significantly attenuated by compartment syndrome (339.2 g, 95% CI, 269.1 to 387.9 g) when compared with the control group (618.7 g, 95% CI, 518.5 to 741.4 g; one-way ANOVA, p = 0.0002; p < 0.01 versus compartment syndrome). As was the case for twitch contraction, pretreatment with vitamin C maintained tetanic contraction (564.1 g, 95% CI, 418.5 to 859 g; one-way ANOVA, p = 0.0002; p < 0.001 versus compartment syndrome alone; Fig. 2b). Tetanic contraction was reduced 24 hours after decompression of compartment syndrome (235.3 g, 95% CI, 170.2 to 281.3) compared with the control group (425.4 g, 95% CI, 395.5 to 515; one-way ANOVA, p = 0.0002; p < 0.001 versus 24-hour compartment syndrome). Preadministration of vitamin C preserved the function of muscle slow fibres 24 hours after injury (431.2 g, 95% CI, 315.1 to 550.1; one-way ANOVA; p = 0.0002; p < 0.01 versus 24 hour compartment syndrome alone; Fig. 2c).

Myeloperoxidase activity. Compartment syndrome induced infiltration of neutrophils into muscle tissue as indicated by a significant increase in the activity of MPO from 6.2 units/g (95% CI, 5.1 to 6.9) in the control group to 10.6 units/g (95% CI, 8.8 to 14.3) in the compartment-syndrome group (one-way ANOVA p = 0.001; p < 0.0001 versus control). This influx of leucocytes and MPO activity were reduced by the administration of vitamin C to 8.6 units/g (95% CI; 7.7 to 9.3; one-way ANOVA; p = 0.0001; p < 0.04 versus compartment syndrome alone; Fig. 2c).

At 24 hours after injury, muscle MPO activity rose from 15.1 units/g (95% CI; 12.5 to 19.2) in the control group to 54.7 units/g (95% CI 34.3 to 74) in the 24-hour compartment-syndrome group; one-way ANOVA, (p = 0.0001; p < 0.001 versus 24-hour control). Pre-administration of vitamin C significantly reduced muscle MPO activity at 24 hours after compartment syndrome (29.4 units/g, 95% CI 20.6 to 41; p = 0.0001, one-way ANOVA; p < 0.05 versus 24-hour compartment syndrome alone; Fig. 2c).

Muscle oedema. This was assessed by the wet-to-dry ratio and was higher in the compartment syndrome group (1.01, 95% CI, 0.94 to 1.08) compared with control animals (0.75, 95% CI, 0.7 to 0.8); one-way ANOVA, p = 0.0017, p < 0.05 versus control.

Oedema was reduced by the pre-administration of vitamin C (0.6, 95% CI 0.32 to 0.88); one-way ANOVA p = 0.0017; p < 0.01 versus compartment syndrome alone; Fig. 2d).

Discussion

Compartment syndrome, which is caused by excessive pressure within a closed fascial tissue compartment, can result from blunt or penetrating trauma, exercise or reperfusion after ischaemia. Increased intracompartmental pressure, either due to increased compartmental contents such as haematoma or reduced compartmental size e.g. as a result of a tight cast, adversely affects neuromuscular function within the compartment by decreasing the available blood supply. Without surgical decompression, this condition leads to ischaemia and death of the affected tissue.

The only established clinical treatment of a compartment syndrome is surgical decompression by fasciotomy. However, despite the availability of pressure-monitoring systems and increased awareness of the condition, compartment syndrome remains a notable cause of morbidity in clinical practice. Establishing a diagnosis of compartment syndrome may be very difficult, particularly in polytrauma-
tised or unconscious patients, and in the very young.25,31,32 In these situations, some authors recommend continuous monitoring of pressure.5 Facilities to perform such monitoring are not universally available or appropriate and a high level of clinical suspicion is critical to making the diagnosis.

Decompression, as a treatment, is not without its complications. Fasciotomy and reperfusion result in further injury to the muscle with an influx of activated neutrophils.8 Subsequent rhabdomyolysis may result in renal failure and acute respiratory distress syndrome.33 Even with adequate surgical decompression, full restoration of muscle and nerve function is not guaranteed. Microvascular dysfunction, a feature of injury to muscle caused by compartment syndrome, is associated with the no-reflow phenomenon and areas of persistent hypoxia within the affected muscle.8 Microvascular dysfunction and no-reflow are probably caused initially by direct oxidant-induced endothelial injury and oedema, and subsequently by neutrophil-mediated injury.34,36 Antioxidant therapies may prevent this endothelial dysfunction and thus allow reperfusion of all of the muscle bed, increasing the likelihood of viability.

The return of muscle blood flow, while essential for the survival of the tissue, initiates a cascade of inflammatory events which cause further tissue injury. The return of oxygen results in a massive increase in the production of free radicals mediated by xanthine oxidase.37,38 These reactive oxygen species, particularly H2O2 directly injure the local endothelium,11,39 and interact with T-lymphocytes to upregulate tumour necrosis factor-α and the production of interleukin-8.40,41 These and other potent cytokines and chemokines attract and activate circulating neutrophils, thus facilitating adhesion and transmigration of neutrophils into ischaemic tissue via the parallel upregulation of adhesion molecules such as ICAM-1.40-44 Neutrophils, in turn, release reactive oxygen species via a membrane NADPH oxidase and MPO, injuring endothelium and tissues further.45 These key steps in the response to muscle hypoxia and the re-establishment of perfusion offer several opportunities for therapeutic interventions.

Vitamin C has been shown to have a number of properties which suggest its potential as a therapeutic agent for the prevention of muscle injury induced by compartment syndrome. Ascorbate is a critical component of the oxidant shield in skeletal muscle, being actively accumulated by muscle endothelium,46 Armour et al11 have shown that ascorbate prevents H2O2-induced endothelial injury in vitro. This scavenging effect on H2O2 which is critical to the recruitment and adhesion of neutrophils,40,41 may explain the reduction in the intramuscular activity of neutrophils as measured by MPO, seen in this experiment. This finding of reduced neutrophil shuttling into the affected tissue may be explained by a reduction in the expression of adhesion molecules. In keeping with the reduction in ICAM-1 upregulation seen in our model Lehr et al12 have also demonstrated that administration of vitamin C reduces oxidant-induced neutrophilic endothelial interaction in vivo. In previous experiments from our group, vitamin C reduced reperfusion-induced skeletal muscle and systemic injury.13,14 Presumably by reducing adhesion-molecule-regulated transmigration of neutrophils and generation of oxidants.

The oral bioavailability of vitamin C makes it suitable for use in clinical practice. Its bioactivity has been demonstrated after oral and intravenous administration.12 However, concerns have been expressed about long-term and high-dose administration of ascorbate. In certain circumstances, vitamin C may show pro-oxidant properties and generate potentially mutagenic lesions.47 However, doses of less than 500 mg per day appear to have an antioxidant effect without significant toxicity48 and achieve high intracellular concentration in circulating neutrophils.10,49 Multiple trials have shown safety and anticarcinogenic properties for doses of vitamin C of between 200 and 400 mg per day.49-51

In conclusion, there is strong experimental evidence for a potential role for antioxidants in the reduction of injury to skeletal muscle caused by compartment syndrome. In this experiment vitamin C reduced ICAM-1 expression and MPO activity in skeletal muscle after compartment syndrome. This reduction in the expression of adhesion molecules and neutrophil infiltration was accompanied by the preservation of the contractile function of muscle and reduction in muscle swelling. The fact that our study assessed the effects of pretreatment with vitamin C allows us only to comment on the potential for such a regime in patients undergoing procedures which carry an appreciable risk of damage to skeletal muscle caused by compartment syndrome. Further study in this model of the effects of the administration of vitamin C in animals after the induction of compartment syndrome could help to ascertain whether vitamin C has a potential therapeutic role in trauma patients with established compartment syndrome.

This study was supported by a research grant from the Cappagh Hospital Trust, Finglas, Dublin 11, Ireland.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References
ORAL VITAMIN C REDUCES THE INJURY TO SKELETAL MUSCLE CAUSED BY COMPARTMENT SYNDROME


