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

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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 at one and 24 hours after decompression of compartment syndrome. Muscle function was 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.

Compartment syndrome is a diagnostic and therapeutic challenge in orthopaedic and trauma surgery. Typical situations which lead to compartment syndrome include crush injuries and revascularisation procedures,[1-3] but it occurs most frequently after injury to the lower limbs in young men.[4] It has been reported as a complication in up to 4% of all tibial fractures,[5] and surgical decompression by fasciotomy remains the only effective treatment. After a fracture or a crush injury, the pressure within the closed fascial compartment rises and is exacerbated further by the accompanying tissue oedema. This increase in pressure may cause venous occlusion or microvascular shutdown, both of which result in microvascular hypoxia.[6,7] Reperfusion results in the generation of large quantities of reactive oxygen species in the hypoxic tissue. Endothelial adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) are upregulated and interact with pro-inflammatory cytokines to chemoattract circulating neutrophils.[8] Although the tissue insult generated by the ischaemia-reperfusion process is an important component of injury to muscle caused by compartment syndrome, the amount of muscle damage in compartment syndrome is greater than that which occurs after an equivalent period of ischaemia alone. This suggests that there is a synergistic effect of increased pressure and sustained microvascular hypoxia on muscle injury in compartment syndrome.[9] Vitamin C is a powerful endogenous antioxidant which is taken up preferentially by circulating neutrophils and lymphocytes.[10] It has been shown to reduce many components of neutrophil-mediated tissue injury after ischaemia-reperfusion injury. In particular, vitamin C can protect the endothelium from direct injury by oxidants, including H_2O_2, and prevent microvascular dysfunction.[11,12] We have demonstrated previously that pretreatment with oral vitamin C reduced lung injury after reperfusion injury of the lower torso and acute reperfusion-induced muscle injury.[13,14] We and others have also shown that the production of oxidants in neutrophils, a key component of neutrophil-mediated cell toxicity in ischaemia-reperfusion injury, is also reduced by the administration of vitamin C.[13,15,16] Our aim in this study was to investigate whether pretreatment with oral vitamin C reduced skeletal muscle injury resulting from compartment syndrome.

Materials and Methods

We used 36 Sprague-Dawley rats weighing 300 to 400 g. They were randomised to receive

Table I. Details of the experimental groups and their treatment

Group	Pretreatment	Description procedure
Acute (3-hour assessment)		
Surgical control (n = 6)	None	Orchidectomy alone
Compartment syndrome alone (n = 6)	None	Orchidectomy, 3-hour compartment syndrome and 1-hour reperfusion
Compartment syndrome and vitamin C (n = 6)	Vitamin C 2g/kg/day for 5 days	Orchidectomy, 3-hour compartment syndrome and 1-hour reperfusion
Delayed (24-hour assessment)		
24-hour post-surgical control (n = 6)	None	Orchidectomy alone
24-hour post-compartment syndrome alone (n = 6)	None	Orchidectomy, 3-hour compartment syndrome and 24-hour reperfusion
24-hour post-compartment syndrome and vitamin C (n = 6)	Vitamin C 2g/kg/day for 5 days	Orchidectomy, 3-hour compartment syndrome and 24-hour reperfusion

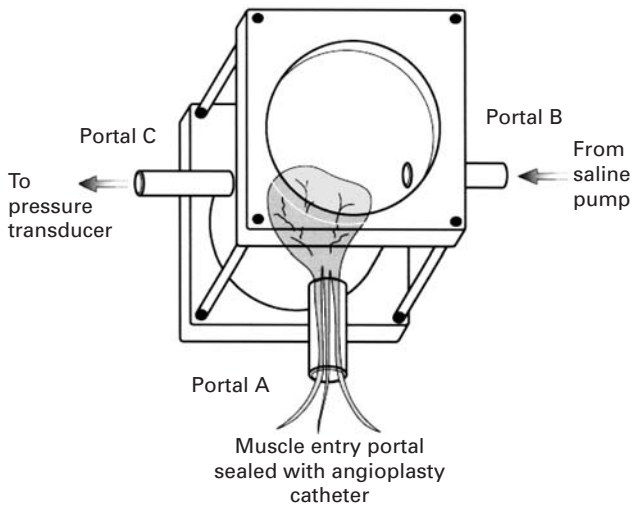

Fig. 1

Diagram of the chamber for the induction of compartment syndrome in rat cremasteric muscle.

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.[14] 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.[6] 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,[14] 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 O_2 saturation were maintained by constant aeration with a 95%O_2/5%CO_2 gas mixture (BOC Gases, Dublin, Eire). The muscle was stimulated using supramaximal pulses (20 V, 2 msec square-wave duration[17-19] 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.[20] The activity of MPO was assayed spectrophotometrically (450 nm, Beckton-Dickinson, Mountain View, California) in weighed homogenised sections of cremasteric muscle as previously described.[14] One unit of MPO was defined as that which degraded 1 μmol of H_2O_2 per minute at 25°C.[20]

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

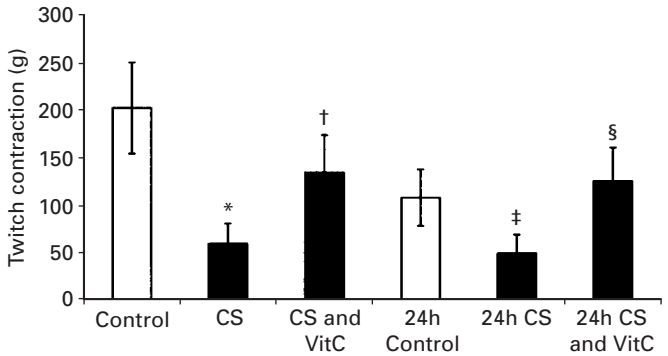

Fig. 2a

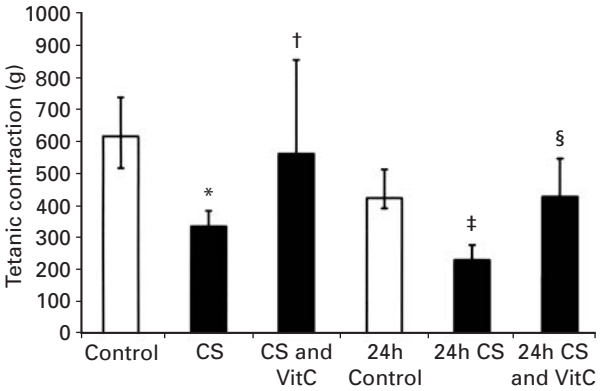

Fig. 2b

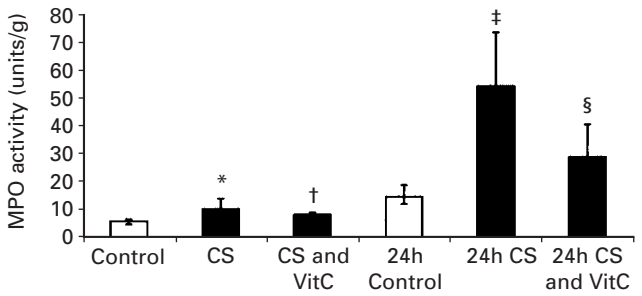

Fig. 2c

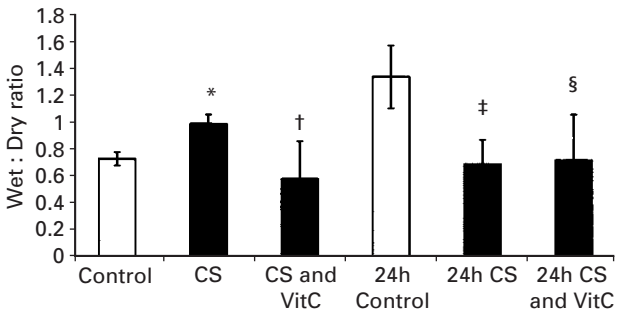

Fig. 2d

Effect of compartment syndrome and pretreatment with vitamin C on a) peak twitch contraction in cremasteric muscle, b) maximum tetanic contraction in cremasteric muscle, c) MPO activity and d) on skeletal muscle oedema. The data are expressed as the mean (95% CI; * $p < 0.05$ *versus* surgical control; † $p < 0.001$ *versus* compartment syndrome alone; ‡ $p < 0.01$ *versus* 24-hour post-surgical control (one-way ANOVA); § $p < 0.01$ *versus* 24-hour post compartment syndrome alone (one way ANOVA). CS, compartment syndrome, VitC, vitamin 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 semiquantative 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). This effect was blunted by pretreatment with vitamin C (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). Twenty-four hours after decompression muscle

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; 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, $p = 0.006$; $p < 0.001$ *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, $p = 0.0002$; $p < 0.001$ *versus* compartment syndrome). As was the case for twitch contraction, pretreatment with vitamin C maintained tetanic contraction (564.1, 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, 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. 2b).

Myeloperoxidase activity. Compartment syndrome induced infiltration of neutrophils into muscle tissue as indicated by a significant rise 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).

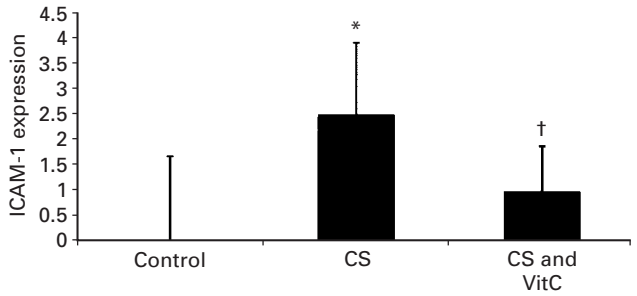

Fig. 3

Effect of compartment syndrome and pretreatment with vitamin C on ICAM-1 expression (data are expressed as the median (95% CI); $p < 0.05$ *versus* surgical control; † $p < 0.05$ *versus* compartment syndrome alone (Kruskal-Wallis test); CS, * compartment syndrome; VitC, vitamin C).

After 24 hours, muscle subjected to compartment syndrome showed a decreased wet-to-dry ratio when compared with the control group (0.71, 95% CI 0.52 to 0.89); *versus* 1.36, 95% CI 1.13 to 1.6); one-way ANOVA, $p = 0.0005$; $p < 0.01$ *versus* 24-hour control). The vitamin-C pretreated group showed decreased oedema *versus* the control group (0.74, 95% CI 0.4 to 1.1); one-way ANOVA, $p = 0.0005$; $p < 0.01$ *versus* 24-hour control), but did not differ significantly from 24-hour compartment syndrome animals ($p = 0.86$; Fig. 2d).

ICAM-1 expression. In cremasteric muscle subjected to compartment syndrome this was significantly increased (2.5, 95% CI 1.4 to 3.94) as compared with the control group (0.0, 95% CI, -0.15 to 1.7; Kruskal-Wallis test, $p = 0.0225$; Mann-Whitney U test; $p = 0.028$). Pre-administration of vitamin C significantly reduced muscle expression of ICAM-1 after compartment syndrome (1.0, 95% CI, 0.12 to 1.9); Kruskal-Wallis test, $p = 0.0225$; Mann-Whitney U test; $p = 0.043$ *versus* compartment syndrome (Fig. 3).

Discussion

Compartment syndrome, which is caused by excessive pressure within a closed fascial tissue compartment,[23] can result from blunt or penetrating trauma, exercise or reperfusion after ischaemia.[3,24,25] 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.[26,27] Without surgical decompression, this condition leads to ischaemia and death of the affected tissue.[28]

The only established clinical treatment of a compartment syndrome is surgical decompression by fasciotomy.[29,30] 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 H_2O_2 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 al[11] have shown that ascorbate prevents H_2O_2-induced endothelial injury in vitro. This scavenging effect on H_2O_2 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 al[12] 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 biovailability 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 toxicity[48] 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 neutrophilic 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.

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References

1. **Hargens AR, Mubarak SJ.** Current concepts in the pathophysiology, evaluation, and diagnosis of compartment syndrome. *Hand Clin* 1998;14:371-83.
2. **Mubarak S, Owen CA.** Compartmental syndrome and its relation to the crush syndrome: a spectrum of disease: a review of 11 cases of prolonged limb compression. *Clin Orthop* 1975:81-9.
3. **Jensen SL, Sandermann J.** Compartment syndrome and fasciotomy in vascular surgery: a review of 57 cases. *Eur J Vasc Endovasc Surg* 1997;13:48-53.
4. **McQueen MM, Gaston P, Court-Brown CM.** Acute compartment syndrome: who is at risk? *J Bone Joint Surg [Br]* 2000;82-B:200-3.
5. **McQueen MM, Court-Brown CM.** Compartment monitoring in tibial fractures: the pressure threshold for decompression. *J Bone Joint Surg [Br]* 1996;78-B:99-104.
6. **Clayton JM, Hayes AC, Barnes RW.** Tissue pressure and perfusion in the compartment syndrome. *J Surg Res* 1977;22:333-9.
7. **Heppenstall RB, Sapega AA, Izant T, et al.** Compartment syndrome: a quantitative study of high-energy phosphorous compounds using 31P-magnetic resonance spectroscopy. *J Trauma* 1989;29:1113-19.
8. **Sadasivan KK, Carden DL, Moore MB, Korthuis RJ.** Neutrophil mediated microvascular injury in acute, experimental compartment syndrome. *Clin Orthop* 1997: 206-15.

9. **Heppenstall RB, Scott R, Sapega A, Park YS, Chance B.** A comparative study of the tolerance of skeletal muscle to ischemia: tourniquet application compared with acute compartment syndrome. *J Bone Joint Surg [Am]* 1986;68-A:820-8.
10. **Rumsey SC, Levine M.** Absorption, transport, and disposition of ascorbic acid in humans. *J Nutr Biochem* 1998;9:116-30.
11. **Armour J, Tyml K, Lidlington D, Wilson JX.** Ascorbate prevents microvascular dysfunction in the skeletal muscle of the septic rat. *J Appl Physiol* 2001;90:795-803.
12. **Lehr HA, Frei B, Olofsson AM, Carew TE, Arfors KE.** Protection from oxidized LDL-induced leukocyte adhesion to microvascular and macrovascular endothelium in vivo by vitamin C but not by vitamin E. *Circulation* 1995;91:1525-32.
13. **Kearns SR, Kelly CJ, Barry M, et al.** Vitamin C reduces ischaemia-reperfusion-induced acute lung injury. *Eur J Vasc Endovasc Surg* 1999;17:533-6.
14. **Kearns SR, Moneley D, Murray P, Kelly C, Daly AF.** Oral vitamin C attenuates acute ischaemia-reperfusion injury in skeletal muscle. *J Bone Joint Surg [Br]* 2001; 83-B:1202-6.
15. **Herbaczynska-Cedro K, Wartanowicz M, Panczenko-Kresowska B, et al.** Inhibitory effect of vitamins C and E on the oxygen free radical production in human polymorphonuclear leucocytes. *Eur J Clin Invest* 1994;24:316-19.
16. **Herbaczynska-Cedro K, Klosiewicz-Wasek B, Cedro K, et al.** Supplementation with vitamins C and E suppresses leukocyte oxygen free radical production in patients with myocardial infarction. *Eur Heart J* 1995;16:1055-9.
17. **Taylor RG, Fowler WM Jr.** Fast and slow skeletal muscles: simultaneous in vitro study. *Arch Phys Med Rehabil* 1976;57:223-8.
18. **Hill DK.** Resting tension and the form of the twitch of rat skeletal muscle at low temperature. *J Physiol (Lond)* 1972;221:161-71.
19. **Close R, Hoh JF.** The after-effects of repetitive stimulation on the isometric twitch contraction of rat fast skeletal muscle. *J Physiol* 1968;197:461-77.
20. **Laight DW, Lad N, Woodward B, Waterfall JF.** Assessment of myeloperoxidase activity in renal tissue after ischaemia/reperfusion. *Eur J Pharmacol* 1994;292:81-8.
21. **Stokes KTY, Abdih HK, Kelly CJ, Redmond HP, Bouchier-Hayes DJ.** Thermotolerance attenuates ischemia-reperfusion induced renal injury and increased expression of ICAM-1. *Transplantation* 1996;62:1143-9.
22. **Nakai K, Traynor FF, Isobe M.** Time course and localization of intercellular adhesion molecule-1 induction in kidney allografts in mice. *Transplant Proc* 1994;26:349-53.
23. **Matsen FA 3rd.** Compartmental syndrome: an unified concept. *Clin Orthop* 1975;113: 8-14.
24. **Allen MJ.** Compartment syndromes of the lower limb. *J R Coll Surg Edinb* 1990;35 (Suppl 6):33-6.
25. **Bourne RB, Rorabeck CH.** Compartment syndromes of the lower leg. *Clin Orthop* 1989;240:97-104.
26. **Hargens AR, Romine JS, Sipe JC, et al.** Peripheral nerve-conduction block by high muscle-compartment pressure. *J Bone Joint Surg [Am]* 1979;61-A:192-200.
27. **Hargens AR, Schmidt DA, Evans KL, et al.** Quantitation of skeletal-muscle necrosis in a model compartment syndrome. *J Bone Joint Surg [Am]* 1981;63-A:631-6.
28. **Heppenstall RB, Sapega AA, Scott R, et al.** The compartment syndrome: an experimental and clinical study of muscular energy metabolism using phosphorus nuclear magnetic resonance spectroscopy. *Clin Orthop* 1988;226:138-55.
29. **Sheridan GW, Matsen FA.** Fasciotomy in the treatment of the acute compartment syndrome. *J Bone Joint Surg [Am]* 1976;58-A:112-15.
30. **Rorabeck CH.** The treatment of compartment syndrome of the leg. *J Bone Joint Surg [Br]* 1984;66-B:93-7.
31. **Whitesides TE Jr, Haney TC, Morimoto K, Harada H.** Tissue pressure measurements as a determinant for the need of fasciotomy. *Clin Orthop* 1975;113:43-51.
32. **Willis RB, Rorabeck CH.** Treatment of compartment syndrome in children. *Orthop Clin North Am* 1990;21:401-12.
33. **Shaw CJ, Spencer JD.** Late management of compartment syndromes. *Injury* 1995; 26:633-5.
34. **Carden DL, Smith KL, Korthuis RJ.** Neutrophil-mediated microvascular dysfunction in postischemic canine skeletal muscle. *Circ Res* 1990;66:1436-44.
35. **Jerome SN, Akimitsu T, Korthuis RJ.** Leukocyte adhesion, edema, and development of post-ischaemic capillary no-reflow. *Am J Physiol* 1994;267:1329-36.
36. **Korthuis RJ, Granger DN, Townsley MI, Taylor AE.** The role of oxygen-derived free radicals in ischaemia-induced increase in canine skeletal muscle permeability. *Circ Res* 1985;57:599-609.
37. **McCord JM.** Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med* 1985;312:159-63.
38. **Welbourn CR, Goldman G, Paterson IS, et al.** Pathophysiology of ischaemia reperfusion injury: central role of the neutrophil. *Br J Surg* 1991;78:651-5.
39. **Weiss SJ, Young J, LoBuglio AF, Slivka A, Nimeh NF.** Role of hydrogen peroxide in neutrophil-mediated destruction of cultured endothelial cells. *J Clin Invest* 1981; 68:714-21.
40. **Kokura S, Wolf RE, Yoshikawa T, Granger DN, Aw TY.** Postanoxic T lymphocute-endothelial cell interactions induce tumor necrosis factor-alpha production and neutrophil adhesion: role of very late antigen-4/vascular cell adhesion molecule-1. *Circ Res* 2000;86:1237-44.
41. **Kokura S, Wolf RE, Yoshikawa T, Granger DN, Aw TY.** T-lymphocyte-derived tumor necrosis factor exacerbates anoxia-reoxygenation-induced neutrophil-endothelial cell adhesion. *Circ Res* 2000;86:205-13.
42. **Gaines GC, Welborn MB 3rd, Moldawer LL, et al.** Attentuation of skeletal muscle ischemia/reperfusion injury by inhibition of tumor necrosis factor. *J Vasc Surg* 1999;29:370-6.
43. **Ioculano M, Altavilla D, Squadrito F, et al.** Tumour necrosis factor mediates E-selectin production and leukocyte accumulation in myocardial ischaemia-reperfusion injury. *Pharmacol Res* 1995;31:281-8.
44. **Rahman A, Kefer J, Bando M, Niles WD, Malik AB.** E-selectin expression in human endothelial cells by TNF-alpha-induced oxidant generation and NF-kappaB activation. *Am J Physiol* 1998;275:533-44.
45. **Weiss SJ.** Tissue destruction by neutrophils. *N Engl J Med* 1989;320:365-76.
46. **Wilson JX, Dixon SJ, Yu J, Nees S, Tyml K.** Ascorbate uptake by microvascular endothelial cells of rate skeletal muscle. *Microcirculation* 1996;3:211-21.
47. **Podmore ID, Griffiths HR, Herbert KE, et al.** Vitamin C exhibits pro-oxidant properties. *Nature* 1998;392:559.
48. **Bendich A, Langseth L.** The health effects of vitamin C supplementation: a review. *J Am Coll Nutr* 1995;14:124-36.
49. **Levine M, Conry-Cantilena C, Wang Y, et al.** Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci USA* 1996;93:3704-9.
50. **Mirvish SS.** Effects of vitamins C and E on N-nitroso compound formation, carcinogenesis, and cancer. *Cancer* 1986;58 (8 Suppl):1842-50.
51. **Gey KF.** Vitamins E plus C and interacting conutrients required for optimal health: a critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancer. *Biofactors* 1998;7:113-74.