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Effects of hyperbaric oxygen on the replanted extremity subjected to prolonged warm ischaemia

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Hyperbaric oxygen; Ischaemia reperfusion; Microsurgery; Rat hindlimb; Replantation

Summary
In this investigation, the influence of hyperbaric oxygen (HBO) therapy on the survival of a replanted extremity subjected to prolonged warm ischaemia is evaluated. Among the relative contraindications to replantation are prolonged warm ischaemia time, since an obstruction to blood reflow ('no-reflow phenomenon') may occur in amputated parts that are subjected to more than 6 h of warm ischaemia.

Twenty-three rat hindlimbs were amputated and subjected to 4 h of normothermic ischaemia. The average weight of the animals was 500 gm, and re-plantation of the hindlimb was performed by bone fixation followed by microvascular anastomosis of the femoral vessels. Limb re-vascularisation was confirmed at the end of all procedures by the milk test, clinical assessment and pulse oximetry recordings (>90%). Eleven animals served as a control group and no further therapy was instituted, whereas 12 animals served as the study (replantation) group and were subjected to HBO therapy for 3 days postoperatively. The therapy was conducted in a large animal chamber for 90 min at 2.5 ata. Limb survival was assessed by capillary refill upon compression, skin turgor assessment and colour. Confirmation of clinical findings was conducted with daily pulse oximetry readings of >90%. Animals were followed up for 7 days at which point all animals were euthanised or were euthanised earlier if a non-viable limb was present.

Two of the 11 limbs in the control group survived following re-plantation, whereas eight of the 12 limbs in the experimental HBO group survived at least 7 days following replantation. This difference was statistically significant (p = 0.0361) using chi-square analysis and Fisher’s exact test.

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Replantation describes the re-attachment of a completely amputated part by restoration of the arterial inflow and venous outflow. The first successful re-plantation of an upper arm amputation was by Malt and MacKann in 1962. In general, any patient with an amputation involving the upper extremity can be considered for re-plantation, but optimal candidates are sharp, guillotine-type amputations of the hand, wrist, forearm or arm that are minimally contaminated. Surgical re-plantation of limbs has developed to the point of technical consistency, with reported patency rates between 85% and 90%. Technically perfect microvascular anastomoses, however, do not always ensure successful re-plantation emphasising the role of other factors on the ultimate outcome. Prolonged warm ischaemia time is considered a relative contraindication to re-plantation. Obstruction to blood reflow may occur in amputated parts that are subjected to more than 6 h of warm ischaemia or 24 h of cold ischaemia. This inability to reperfuse the tissue has been termed the ‘no-reflow phenomenon’. Muscle is the one tissue most susceptible to ischaemia and begins to undergo irreversible changes after 6 h at room temperature. Because proximal forearm or upper arm amputations contain a large muscle mass, it is vitally important that such amputated parts be cooled as quickly as possible and, if necessary, reperfused through arterial shunts to reduce the warm and cold ischaemia times if re-plantation is to be successful. Because the digits do not contain muscle, they have much longer ischaemic tolerance; however, questions remain with regards to the optimal storage of amputated tissue awaiting re-plantation and whether therapy in the post-replantation period can increase re-plant survival. Presently, most parts are simply cooled after amputation, thereby decreasing tissue metabolism, and allowing increased survival of re-planted parts. Various animal models have been used to study the no-reflow phenomenon. In one study performed on 25 amputated rat hindlimbs replanted after various periods of warm ischaemia, the incidence of limb failure was found to be 0% after 2 or 3 h of warm ischaemia, 50% after 4 h and 80% after 5 h. A duration of 4 h of warm ischaemia was selected for our study to evaluate the influence of hyperbaric oxygen (HBO) therapy on the rat hindlimb following re-plantation. This is to serve as the equivalent of 6 h of warm ischaemia in an amputated human part.

The use of HBO therapy administered to the amputated part during storage (pre-replant) has been shown to be beneficial to re-plant survival. HBO therapy increases the tolerance of tissue to ischaemia and improves its survival possibility; in addition, it is thought to protect the microcirculation from reperfusion injury. As the inspired partial pressure of oxygen (pO2) is increased to 3 ata, the dissolved oxygen is sufficient to support tissue in which no functioning haemoglobin is available. This becomes essential as red blood cell sludging and thrombosis occurs in reperfusion injury immediately after re-plantation. HBO therapy has also been shown to increase the survival of free tissue transfer from 10% to 60% by preservation of the tissue prior to transplantation in rats.

We hypothesised that the administration of HBO post reperfusion (post replant) may decrease reperfusion injury after re-plantation and increase survival following prolonged warm ischaemia. If this hypothesis is supported, HBO could be applied clinically to help ameliorate the ill effects of reperfusion injury on the re-plantations with hopes of either increasing survival of limbs subjected to prolonged warm ischaemia or extending the indications for re-plantation following prolonged ischaemia.

Material and methods

The experimental design and protocol was conducted in accord following approval by the Institutional Animal Care and Use Committee (IACUC). Twenty-three Sprague-Dawley male rats (Rattus norvegicus) which weighed an average 500 g (range: 470–590 g) were included in the study analysis. Fifteen rats were excluded from the evaluation as they were used for technique refinement or auto-cannibalised their limb prior to use of the Elizabethan collar.

Anaesthesia protocol

Operative anaesthesia was induced with the intramuscular administration of 35 mg kg⁻¹ ketamine and 5 mg kg⁻¹ xylazine. Inhalational anaesthesia was maintained with a mixture of 50% isoflurane and oxygen delivered via a rodent anaesthesia device. Postoperative pain was managed with the administration of subcutaneous buprenorphine, 0.05 mg kg⁻¹ (twice daily).

Surgical procedure

After the animals were placed under anaesthesia, the left hindlimb was shaved and surgical preparation was performed. A circumferential incision was made at the mid-thigh region and extended into the skin and the underlying musculature. The inferior epigastric vessels were isolated and cauterised. The femoral nerve and femoral vessels were isolated and divided using a microscope. Microvascular clamps were applied proximally on the isolated femoral artery and vein prior to division. This time point was marked as the beginning of ischaemia time (T₀). Then, the proximal to mid-portion of the femur was exposed in the subperiosteal plane. The femur was transected with
a power-driven saw burr tip (Stryker, Kalamazoo, MI) and subsequently shortened by 1 cm to decrease vessel tension upon replantation. The amputated leg was wrapped in gauze moistened with normal saline and placed in a sterile, plastic, sealed container at room temperature (68°F; Figures 1 and 2). The replantation procedure was begun approximately 2 h after the initiation of ischaemia time (T₀) prior to the 4-h total warm ischaemia time. The animals were kept under anaesthesia during the entire period of amputation, ischaemia and replantation. The replantation aspect of the procedure was begun with the fixation of the amputated femur to the residual proximal femur performed in a retrograde fashion through the knee with a 0.045-inch Kirschner wire (Figure 3). The femoral artery and vein were then anastomosed with the aid of the operative microscope (10–25×) in an interrupted fashion using 10/0 nylon. All anastomoses were performed under the supervision of the surgeon (first author). Vessel clamps were removed and anastomotic patency observed and confirmed using the milk test for 20 min prior to skin closure. The timing of re-vascularisation was synchronised such that the total ischaemia time was approximately 4 h in all cases. The muscular layer was sutured circumferentially, and this was followed by skin closure. An effort was made to close the limb muscle and skin layer with the hip in an adducted and flexed position in order to minimise tension on the anastomosis. An Elizabethan collar was used around the rats’ neck to allow them to consume unlimited food and water yet to prevent auto-cannibalisation of the replanted limb—a phenomenon which was observed in several refinement animals. The rats were caged separately—immediately and for the duration of the experiment after surgery (7 days). The animals were assessed in the immediate postoperative period and then daily with pulse oximetry recordings of the replanted and contralateral limb, as well as by clinical examination for the next 7 days to assess limb viability. A pulse oximetry value of less than 90% was consistent with a non-viable limb in all cases. The effect of post-replantation HBO treatment was analysed by comparing the experimental group treated with HBO to a control group treated with the identical amputation/ischaemia/replantation protocol, but without postoperative HBO therapy.
Group 1 (control)

The surgical procedure outlined above was performed on 11 control animals and no further therapy was instituted. The animals were assessed postoperatively with pulse oximetry recordings of the replanted and contralateral limb and clinically for the next 7 days to assess limb viability.

Group 2 (HBO therapy post replantation)

The same surgical procedure outlined above was performed on 12 animals. Immediately after replantation, this group of rats was subjected to 90 min of HBO therapy in a large, animal, hyperbaric chamber (2.8 ata) once they recovered from surgery on the day following replantation and for 90 min 2 times a day for 3 days postoperatively. A 5-min air break was given to the animals after 45 min into each dive. The animals were assessed postoperatively with pulse oximetry recordings of the replanted and contralateral limb and clinically for the next 7 days to assess limb viability. If limb non-viability was noted prior to 7 days, the animal was electively euthanised. All animals were euthanised 7 days following replantation.

Statistical analysis

Statistical analysis was performed with a chi-square analysis and Fisher’s exact test using statistical software (StatView and JMP, SAS, Cary, NC, USA).

Results

Twenty-three rats were included in the experimental and control groups, consisting of 12 rats in the experimental hyperbaric group and 11 rats in the control group. The average femoral artery and vein diameters were 1 and 1.2 mm, respectively, in both groups. Limb survival was assessed clinically with capillary refill upon compression, skin turgor assessment and colour. Clinical assessment was confirmed with pulse oximetry where all ‘clinically viable’ limbs had pulse oximetry values >90% at 7 days post replantation.

Two of the 11 limbs in the control group survived following replantation, whereas eight of the 12 limbs in the experimental HBO group survived at least 7 days following replantation (Table 1.) This difference was statistically significant (p = 0.0361) using chi-square analysis and Fisher’s exact test.

We note that, clinically, limbs treated with HBO in the postoperative period appeared to have less oedema than those limbs not treated with HBO therapy post replantation (Figures 4 and 5). Limbs which did not survive were, on average, ’non-viable’ 2–3 days following replantation.

Discussion

A warm ischaemia time greater than 6 h for proximal limb amputations (proximal to the carpus) is considered a relative contraindication for replantation. This recommendation has been based not only on the irreversible necrotic changes seen primarily in the muscular component of the amputated part, but also on the no-reflow phenomenon and

<table>
<thead>
<tr>
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<th>Ischaemia time</th>
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<tr>
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<td>4.0</td>
<td>Did not survive</td>
<td>Replant died POD 1</td>
</tr>
<tr>
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<td>Control</td>
<td>4.0</td>
<td>Did not survive</td>
<td>Replant died POD 3</td>
</tr>
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associated reperfusion injury. We do not have an explanation for the markedly increased limb loss noted in the control group, other than ischaemia–reperfusion injury secondary to prolonged warm ischaemia. Our findings show that replantation of an amputated rat hindlimb may be indicated even after prolonged ischaemia in a warm environment, if adjunctive HBO therapy can be employed in the postoperative period. These effects of HBO on decreasing the sequelae of reperfusion injury in ischaemic tissue continue to be investigated. One might theorise that treating an ischaemic axial skin flap with HBO would increase free-radical production and exacerbate reperfusion injury. Instead, HBO studies have shown an increase in viability following postoperative treatment with HBO of pedicle flaps, island flaps and free flaps in rats, guinea pigs and rabbits. 13,14

HBO therapy has also been experimentally shown to reduce skeletal muscle oedema and necrosis in rat hindlimb tourniquet ischaemia15 and compartment syndrome models in canines. 16 This was consistent with our clinical observations in the replanted hindlimbs in our study that were treated with HBO. In addition, HBO has been shown to delay the progression of metabolic acidosis in an amputated limb model, further supporting the tissue-preserving effect of oxygen when delivered under hyperbaric conditions.17

Zamboni et al. used an in vivo microcirculatory preparation to quantitatively evaluate key morphological changes occurring during reperfusion following 4 h of global ischaemia in the rat gracilis muscle. They showed a significant early and sustained increase in leucocytes adherent to the microvenular endothelium during reperfusion. Leucocyte adherence with subsequent neutrophil activation and liberation of oxygen-derived free radicals results in venular endothelial damage and adjacent arteriolar ‘no-reflow’ vasoconstriction. Acute HBO treatment up to 1 h after reperfusion was beneficial in the acute treatment of skeletal muscle reperfusion injury occurring from limb transplantation following prolonged ischaemia. 18 The focus of future studies would be to further investigate the significant role of HBO in reperfusion following replantation, particularly in limbs subjected to prolonged warm ischaemia, so that clinical applicability in these desperate situations may become a reality.

Financial disclosure and products

None of the authors on this manuscript (Christopher J. Salgado, Amir Jamali, Juan Ortiz, Jason J. Cho, Vincent Battista, Samir Mardini, Hung-Chi Chen and Raoul Gonzales) have any commercial associations that may pose or create a conflict of interest with the information presented in the submitted manuscript. This includes: consultancies, stock ownership or other equity interests, patent-licensing arrangements, and payments for conducting or publicising the study described in the manuscript.

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