Histological and ultra-structural effects of rapid muscle expansion through intramuscular administration of carbon dioxide: an intra-operative study in an animal model


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Abstract. – INTRODUCTION: Carbon dioxide (CO₂) therapy refers to trans-cutaneous or sub-cutaneous administration of CO₂ for therapeutic purposes, and recent studies have pointed out that it produces a vasodilation effect after it is locally injected, which helps amplify the reconstructive potentiality of an expanded-muscle flap.

MATERIALS AND METHODS: Thirty male Wistar rats, weighting between 350 and 400 g, were randomly divided into three groups of 10. In the first group, single intra-operative rapid expansion was carried out under the right latissimus dorsi muscle. In the second group, for five days prior to surgery, a pre-treatment with intramuscular injections of CO₂ was performed. The third group served as controls. For each group, the latissimus dorsi muscle was fixed as soon possible after mice died, and ultrathin sections of it examined with transmission electron microscope.

RESULTS: In the treated group, the majority of expanded muscles showed a normal striation pattern, whereas a few fibers showed mild disorganization of the myo-filaments in the sarcomeres, which appeared overstretched (average 2.37 µm).

CONCLUSIONS: This evidence could demonstrate a greater capacity of muscle recovery after treatment by CO₂ expansion.

Key Words: Carbon dioxide therapy, Tissue expander, Ischemia, Neoangiogenesis.

Introduction

Intra-operative acute tissue expansion is a reliable technique that helps amplify the reconstructive potentialities of a muscle flap normally used for the coverage of wider tissue defects. Although clinical research has confirmed its usefulness, the histological and biochemical mechanisms governing muscular compliance to acute expansion are still not completely understood. In addition, recent studies have pointed out a vasodilatation effect after local injections of carbon dioxide which can be of help when a muscular flap needs to be harvested. In this study, we executed a tissue expansion of the dorsal muscle of male Wistar rats. In order to achieve this tissue expansion, Carbomed equipment was used for the controlled release of medical CO₂ and a daily 5 ml dose was injected. Successively, muscle samples of treated and non-treated mice were examined with the aim of evaluating the biochemical and histological muscular modifications after intra-operative acute expansion. By examining the latissimus dorsi muscle of rats which had been pre-treated with local injections of CO₂, the results suggested that a controlled infusion of CO₂ might improve muscle trophism, thus, facilitating the quality of tissue recovery and ultimately improve therapeutic prospects overall.

Materials and Methods

Surgical Procedures and CO₂ Administration Protocol on an Animal Model

Thirty male Wistar rats, weighting from 350 to 400 g, were randomly divided into three groups of 10 each (group A, B and C). All the animals were kept in cages, and fed with food and water ad libitum. Independently from the group to
The muscle was isolated and dissected, and the tissue was quickly skinned and pinned to a piece of cork in the fully extended position and immediately fixed in 2.5% cacodylate-buffered glutaraldehyde, pH 7.3, at 4°C. After 1 hour, the muscle was minced into approximately 2 mm³ pieces and replaced in the same fixative for a further 3 hours. The specimens were washed overnight in the same buffer, post-fixed in buffered, 1% osmium tetroxide for 2 hours, washed, dehydrated through a graded series of ethanol, and then cleared in propylene-oxide and embedded in Epoxy resin (Araldite).

Semithin sections 1 µm thick were cut with glass knives on an LKB V Ultratome (Washing-ton, DC, USA), stained with 1% toluidine blue and examined under the light microscope for a general evaluation of tissue morphology. Ultra-thin sections from selected areas were cut with a diamond knife using the same ultra-microtome. They were then retrieved and placed on uncoated copper grids, double-stained with uranyl acetate/lead citrate and examined at 100 kV with a Philips 208 S transmission electron microscope.

Statistical Analysis

For each group we measured 75 sarcomeres. The length of sarcomeres was measured using Image J. The differences among groups were analyzed by analysis of variance (Kruskall-Wallis test for non parametric data, \( p < 0.05 \)). The Kruskall-Wallis test was followed by Dunn’s post hoc test.

Results

Transmission Electron Microscopy

Group A (Control group). The muscle fibers of control mice displayed a normal architectural order, with regularly arranged, uniformly sized and shaped myofibrils and peripherally located nuclei. The ultra-structural appearance of the sarcomeres, ranged from 1.5-1.8 µm in length (average 1.60 µm), and were homogenous. Small mitochondria were situated at both sides of Z lines and transverse tubules formed well structured triads with two cisterns of sarcoplasmic reticulum.

The vessels were normally shaped, exhibiting thin endothelial cells encircled by a continuous basement membrane (Figure 2).

Group B (Expansion). The majority of expanded muscles showed a normal striation pattern, whereas few fibers showed a mild disorganiza-
tion of the myo-filaments in their sarcomeres, which appeared overstretched (average 2.37 µm). In these altered areas, empty vacuoles and mitochondria with a swollen appearance were observed interspersed among myofibrils, and mainly in the peri-nuclear region where they formed small clusters. No signs of interstitial fibrosis, necrosis or inflammation were observed. Vascularization had a normal morphology (Figure 2).

Group C (CO₂+ expansion). All the examined specimens exhibited a normal appearance, with sarcomeres organized in regular arrays and peripheral nuclei. The mean length of the sarcomere was 1.9 µm. Most mitochondria were homogeneous in size and shape, but a few showed evidence of swelling, especially the ones adjacent to the nucleus, where some empty peri-nuclear vacuoles were also seen. Micro-vessel density appeared increased and demonstrated a vascular network characterized by an alternation of thick and thin endothelial cells filled with Weibel-Palade bodies. Both basal membrane and intima appeared thickened whilst the pericytes were evident (Figure 2).

**Discussion**

The use of tissue flaps to cover a loss of substance is one of the main principles of reconstructive surgery. Flaps are tissue transplants carried out maintaining the vascular connections with the donor site. Radovan, in 1982, first used
mechanical expansion of tissues for reconstructions, creating a silicone balloon that could be implanted subcutaneously (or even under muscles) and which could be slowly and progressively inflated from the outside by means of water injections. The mechanical push, due to the volumetric increment of the device, was found to be effective in expanding the overlying tissues, thus amplifying their reconstructive potentialities. Subsequently, several measures and shapes of tissue expanders were produced, and they are still successfully used in reconstructive surgery now. Tissue expansion is a safe technique, but it requires two surgical steps, a relatively long expansion time, and has some limits due to the parenteral implant of a foreign body. With regard to the progressive slow expansion of muscular tissues, Kim et al., in a histological analysis of the gracile muscle of rats, found that, after three-weeks of expansions, the normal structural features did not modify, but an increase in the number of sarcomeres in expanded muscular fibres resulted. Conversely, Gur et al., in 1998, found evidence that tissue expansion can lead to mechanical and ischemic injuries. Rapid intra-operative tissue expansion is an acute mechanical expansion of the tissues during the reconstructive act. Similar to slow expansion, it amplifies the reconstructive potentialities of a general muscular flap, in that the coverage of tissue losses are more extended if compared to the flap itself. Although clinical evidence confirms the usefulness of this technique, the histological and biochemical rules of the phenomena leading to muscular adaptability and rapid expansion are still poorly known. Hauben et al. in 2010 created a mechanical device for fast expansions in muscular-cutaneous reconstructions. However, it is important to emphasize that rapid expansion can lead to mechanical damage due to muscle stretching. Furthermore, ischemia may result, caused by traction of tissues in the acute phase of intervention, and may compromise the vitality of the expanded flap. It is well known that tissue expansion always provokes ischemia of variable duration. Prevention of the ischemic effects on acutely expanded tissues can be achieved by some ischemic preconditioning.

The histological analyses of the prepared muscles investigated revealed and quantified the possible tissue injuries and ultra-structural modifications of the muscular compound after expansion. This was demonstrated by evaluating the following parameters: muscular trophism, myofibril aspect, myofibril degeneration and/or regeneration, inflammatory phenomena, increase or lessening of blood vessels, anatomic abnormalities of blood vessels, necrosis.

Clinical studies have pointed out that local administration of CO₂ has positive effects on tissue microcirculation, increasing oxygen partial pressure and vascular wall motility in the treated areas. It is well known that CO₂ provokes vasodilatation and increases oxygen release in tissues (Bohr effect).

In fact, our electron microscopy study of the treated areas has shown that the controlled administration of CO₂ seems to have a positive effect on microcirculation, causing an increased density of the vascular network, as characterized by the alternation of thick and thin endothelial cells filled with Weibel-Palade bodies. Furthermore, both basal membrane and intima appeared thicker than in the two groups where CO₂ was not delivered. These structural changes could contribute to improved tissue oxygenation and, thus, to lower the occurrence of ischemic events during rapid expansion.

The increase in the local microcirculation might explain the better preservation of the structures observed in sarcomeres. After the rapid expansion, in fact, an organization into regular arrays and peripheral nuclei, a lower overstretching, as well as a better preservation of mitochondria was noted in the treated group respect to the other two groups.

This evidence could demonstrate a better tolerance or a greater capacity of recovery after CO₂ expansion.

Conclusions

The controlled administration of carbon dioxide in an animal model, thanks to its proven ability to improve tissue trophism, has a favorable effect on the muscles undergoing a rapid expansion. This feature, no doubt, requires more detailed studies in humans, but could be of major utility if it were to be applied in the surgical field, improving the characteristics of the expanded tissue and whilst reducing the time of expansion required.

Conflict of Interest

The Authors declare that there are no conflicts of interest.
References


