The Effect of Transcutaneous Application of Carbon Dioxide (CO₂) on Skeletal Muscle

INTRODUCTION
The presence of the carbonated spa (carbonated therapy) has been known for a long time in the Europe, and it is known that carbonated therapy is effective against cardiac disease and skin trouble. However there has been no report investigating the effect of the carbonated therapy on muscle.

We hypothesized that the transcutaneous application of CO₂ to muscle exhibited similar effect to the exercise training. In this study, we applied CO₂ to the rat lower limbs transcutaneously, and investigated the effect on the fast muscle (tibialis anterior muscle; TA muscle). The changes of the gene expression and protein in TA muscles were analyzed.

MATERIALS AND METHODS

Experimental set up
The use of animals was approved by the Animal Care and Use Committee of Kobe University Graduate School of Medicine. 14 male Sprague Dawley rats of 5 weeks of age were randomly divided into 2 groups (treatment group (CO₂): transcutaneous CO₂ application; non treatment group (Control)). We used 100% CO₂, and transcutaneous CO₂ absorption enhancing hydrogel (Hydrogel) and CO₂ adapter that seals the body surface and retains the gas inside. Then, the area of skin with Hydrogel was sealed up, and CO₂ gas was flowed into the area. (International patent application number: WO2004/002393)

Procedure for CO₂ treatment
Under pentobarbital anesthesia (6 ml/kg), we cut the hairs of right lower limbs, and apply Hydrogel to these lower limbs. The CO₂ was sealed up, and CO₂ gas was flowed into the area. This treatment was performed twice a week for 12 weeks.

Surgical Procedure
Rats were sacrificed after 12 weeks, and TA muscles of right lower limb were dissected carefully and harvested.

Determination of the relative mitochondrial copy number
Genomic DNA was isolated from a 10 mg transversal slice of midbelly TA using the GenElute Mammalian Genomic DNA Miniprep Kit. Mitochondrial DNA (mtDNA) content relative to peroxisome proliferators-activated receptor (PPAR)-γamma coactivator-1 (PGC-1α) gene was measured using Real-time PCR as described previously [1].

Histchemical staining
Unfixed frozen TA muscles were sectioned at 10 µm thickness in the cryostat and the ATPase staining was performed as previously described [2]. The condition was pH 4.6.

Isolation of myosin heavy chain (MHC)
The protein solution was isolated from a 10 mg transversal slice of midbelly TA using two-dimensional electrophoresis. The MHC analysis was as described previously [3]. After electrophoresis, gels were silver-stained by using a Wako Silver Stain Kit. Images of MHC analysis was taken to be statistically significant. (*, p < 0.05, **, p < 0.01)

RESULTS

TA Muscle Weight (Table 1)
The upward tendency of muscle weight and muscle weight/body weight ratio in CO₂ group was observed (n = 14). Real-time PCR (Figure 1)
The gene expression of PGC-1α and Mitochondria in CO₂ group was significantly increased (n = 12). MHC isoform (Figure 2)
The percentage of IIB fibers was decreased, and those of IID fibers and/or IIA fibers in CO₂ group were increased. The significant difference in the percentages of IIB fibers and IIA fibers between two groups was observed (n = 12).

DISCUSSION
Generally, the muscle fibers transform from IIB fibers to IID fibers and IIA fibers in the aerobic exercise situation, whereas in long term bed rest situation they transform to IIB fibers. The result of our study showed that the muscle fibers in which CO₂ was transcutaneously applied transformed from IIB fibers to IID fibers and/or IIA fibers.

PGC-1α is known to be up-regulated under the aerobic exercise [4]. The muscle fiber transformation mechanism relates to the increase of the mitochondrial quantity. PGC-1α increases the mitochondrial quantity [4]. Our results demonstrated that PGC-1α was up-regulated by transcutaneous CO₂ application, which correspondingly increased the mitochondrial quantity. These data indicated that transcutaneous CO₂ application to TA muscles under the non-exercise conditions may possess similar effect to the aerobic exercise.

The current study suggests that the transcutaneous application of CO₂ may have therapeutic potential for muscular strength recovery of disuse atrophy in post operative patients and elderly people.

REFERENCE