Transcutaneous Carbon Dioxide (CO2) Therapy Suppresses Bone Destruction By Breast Cancer Metastasis With Decreased Expression Of Hypoxia-inducible Factor-1α (Hif-1α) In Vivo.

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Disclosures:

Introduction:
Bone is one of the most common sites of metastasis in breast cancer [1]. Bone metastasis is usually associated with devastating complications, including severe bone pain, pathologic bone fracture which usually needs stabilization with prosthetic implants, hypercalcemia, and nerve compression syndromes, and causes increased morbidity and eventual mortality in breast cancer patients [1]. Hypoxia is a common feature of solid tumors [2]. It has been demonstrated that hypoxia increases hypoxia inducible factors (HIF)-1α, and increased level of HIF-1α is associated with increased metastasis and poor prognosis in breast cancer patients [3]. We have previously shown that a transcutaneous CO2 therapy can improve tumor hypoxia and that the therapy reduces tumor burden and HIF-1α expression in sarcomas [4, 5]. Based on these findings, we hypothesized that our transcutaneous CO2 therapy may inhibit bone destruction by breast cancer cells via decreasing HIF-1α expression. The purpose of this study was to investigate the effects of the transcutaneous CO2 therapy on bone destruction and HIF-1α expression using a mouse model of human breast cancer metastasis to bone.

Methods:
Cells: A human breast cancer cell line, MDA-MB-231 was used in this study.
Animal models: Seventeen BALB/c nude mice, aged 5 weeks, were obtained from CLEA Japan, Inc. (Tokyo, Japan), and MDA-MB-231 cells were injected into the right tibia of anesthetized mice at a dose of 1.0×10⁶ cells in 10μl PBS. Then, mice were randomly divided into two groups; CO2 treated group (n=8) and control group (n=9). Transcutaneous CO2 therapy was performed as previously described (Figure 1) [4]. Control animals were treated similarly, replacing CO2 with room air. Treatment commenced after confirming bone destruction by μCT (R_mCT2; Rigaku, Tokyo, Japan), and was performed twice weekly for 2 weeks. Tumor volume was calculated as previously described [4], and body weight of mice was monitored twice weekly. All animals were sacrificed after treatment. The tibiae were scanned using the μCT, and bone volume of tibiae was calculated. Each tibia was excised, fixed, and decalcified.

Immunohistochemical analysis: The sections of tibiae were immunohistochemically stained with a HIF-1α antibody. Sections were also stained with haematoxylin-eosin (H&E) and evaluated microscopically to confirm the presence of bone destruction. The data was analyzed statistically using student’s t-test. p<0.05 was considered to be significant.

Results:
At the end of the study, the tumor volume in the CO2 treated group was significantly smaller compared with the control group (P<0.05, Figure 2). The μCT images showed that bone destruction was strongly suppressed in the CO2 treated group, whereas it was extensively observed in the control group (Figure 3). The bone volume in the CO2 group was significantly
preserved compared with the control group (p<0.05, Figure 4). The relative bone volume was decreased by 30% in the control group, whereas it was decreased only by 10% in the CO2 treated group. These results indicate that bone destruction caused by breast cancer cells was significantly suppressed in the CO2 treated group. Additionally, in the CO2 treated group, HIF-1α-positive cells were hardly observed, conversely, immunostaining for HIF-1α was extensively positive in the control group (Figure 5). Transcutaneous CO2 therapy did not cause any observable negative side effects in terms of body weight loss in mice.

**Discussion:**
We have previously demonstrated that a transcutaneous CO2 therapy can improve hypoxic condition in sarcomas, and can reduce tumor burden and lung metastasis [4, 5]. Recently, Dunn et al. have reported that inhibition of HIF-1α significantly decreased the extent of osteolysis at the metastatic site of breast cancer [6]. In the current study, we examined the effect of transcutaneous CO2 therapy on osteolytic bone destruction caused by breast cancer cells, and on hypoxic condition in vivo. The bone volume in the CO2 treated groups was preserved compared with that in the control group. This result suggests that transcutaneous CO2 therapy inhibits bone destruction by breast cancer metastasis. Moreover, HIF-1α expression in the metastatic site in the CO2 treated group was suppressed than that in the control group. These results suggest that our transcutaneous CO2 system could suppress bone destruction by breast cancer metastasis via improving hypoxic condition. This study may imply that a transcutaneous CO2 therapy can bring a therapeutic breakthrough for osteolytic bone destruction caused by cancer metastasis.

**Significance:**
Transcutaneous CO2 application reduced bone destruction caused by breast cancer metastasis with improved hypoxic condition in vivo. The CO2 therapy may be a therapeutic breakthrough for metastatic bone destruction.

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**References:**
Transcutaneous CO2 therapy on an animal model. The area of skin around the implanted tumor is covered with CO2 hydrogel and sealed with a polyethylene bag, through which CO2 gas is administered.

Effect of transcutaneous CO2 therapy on tumor volume.
The μCT images.

(a) 3D CT  (b) Sagittal section  (c) Coronal section

Control

CO₂

Figure 4

Bone volume after treatment.

* N.S.

Bone volume (mm³)

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<th>Control</th>
<th>CO₂</th>
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* p<0.05
Figure 5

Expression of HIF-1α

Joint cartilage

Tumor

Control

CO₂

Immunohistochemical staining of HIF-1α in the treated bone.

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