Inhibition of Growth in a Rabbit VX2 Thigh Tumor Model with Intraarterial Infusion of Carbon Dioxide–Saturated Solution

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ABSTRACT

Purpose: To evaluate the efficacy of intraarterial infusion of CO_2-saturated solution in rabbit VX2 thigh tumors.

Materials and Methods: Fourteen Japanese white rabbits had VX2 tumors implanted in the right femoral muscle 3 weeks before intraarterial infusion. Rabbits were divided into control and CO_2 groups (n = 7 each). Fifty milliliters of solution (saline solution and CO_2-saturated solution for the control and CO_2 groups, respectively) was administered via a 24-gauge catheter in the ipsilateral iliac artery close to the feeding artery of the VX2 tumor. All rabbits were killed for tumor harvest on day 3 after the procedure. Tumor volume was evaluated with in vivo direct caliper measurement and contrast-enhanced computed tomography (CT). Tumor apoptotic changes were examined by DNA fragmentation assay and immunoblot analysis. The tumor growth ratio and apoptotic cell rate were analyzed.

Results: Body weight was equally increased in both groups, but the mean tumor growth ratio was significantly decreased in the CO_2 group compared with the control group (−9.5% ± 7.9 vs 27.2% ± 6.6 and 4.1% ± 4.4 vs 35.7% ± 4.5 measured by calipers and contrast-enhanced CT, respectively; $P < .01$). Apoptotic activity in the CO_2 group was higher than in the control group (number of apoptotic cells per area, 215.0 ± 58.7 vs 21.8 ± 5.4; adjusted relative density of cleaved caspase-3, 0.23 ± 0.07 vs 0.04 ± 0.01; $P < .01$).

Conclusions: Intraarterial infusion of CO_2-saturated solution inhibits rabbit VX2 thigh tumor growth by activation of apoptotic cell death through cleaved caspase-3 upregulation.

ABBREVIATIONS

H&E = hematoxylin and eosin, MFH = malignant fibrous histiocytoma, pO_2 = O_2 pressure, SE = standard error

Carbon dioxide gas is often applied as negative contrast medium in angiography. CO_2 is particularly used in patients with allergies to contrast media or renal dysfunction because the difference in X-ray permeability between CO_2 gas and blood can be visualized by digital subtraction angiography. The infused CO_2 gas dissolves in blood immediately, and few side effects occur in the human body (1–3). Carbonated spas are beneficial and CO_2 therapy is effective in treating skin problems (4–6). The mechanisms of this beneficial effect are an increase in blood flow and microcirculation, nitric oxide–dependent neocapillary formation, and a partial increase in O_2 pressure (pO_2) in local tissue known as the Bohr effect (7).

In terms of tumor growth, contradicting findings of the effects of CO_2 have been reported. A previous report (8) showed that CO_2 exposure increases the growth of

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human ovarian carcinoma cells in vitro (8). However, a recent in vitro study demonstrated that CO_2 induces cell death through the mitochondrial pathway in human neuroblastoma cells (9). An in vivo study (10) also showed that transcutaneous application of CO_2 may contribute to inhibition of tumor growth in human breast cancer and malignant fibrous histiocytoma (MFH) by inducing apoptotic change through the upregulation of mitochondrial function. However, this study targeted superficial tumors (10), but the majority of tumors are located in deep tissue.

Minimally invasive interventional drug delivery methods, such as transcatheter arterial chemoembolization, radioembolization, and immunoembolization, are emerging as viable treatment options for human cancer with the advantage of a decrease in normal organ toxicity (11). Intraarterial CO_2-saturated solution infusion may have tumoricidal effects on deep-tissue tumors as well. Therefore, we performed a study on rabbit VX2 thigh tumors to evaluate the effect of intraarterial CO_2-saturated solution infusion on tumor growth and apoptotic pathways. Moreover, its safety for future development as a new treatment option in human cancer was evaluated.

MATERIALS AND METHODS

VX2 Animal Model

Fourteen healthy female Japanese white rabbits were used in the experiments, which were approved by the ethics committee of the laboratory animal institution at the study university. All rabbits (approximately 3–4 months of age; weight, 2.3–2.9 kg) were implanted with fresh VX2 tumors by injecting 0.1 mL VX2 tumor tissue suspension into the femoral muscle tissue of the proximal right thigh (provided by Japan SLC, Shizuoka, Japan) 3 weeks before intraarterial infusion. Subsequently, the rabbits were randomly divided into two groups: the CO_2 group (n = 7) and the control group (n = 7).

Preparation of CO_2-Saturated Solution

A 24-gauge needle (Neolus; Terumo, Tokyo, Japan) was inserted into a 100-mL bottle of saline solution with the tip inside the solution, and connected to a CO_2 gas cylinder through an extension tube. For air outflow, a 26-gauge needle (Neolus; Terumo) was inserted into the bottle with the tip above the solution. CO_2-saturated solution was prepared by dissolving CO_2 gas into the saline solution for 10 minutes at 25°C. CO_2 saturation was determined by setting the acidity (ie, pH) value of the saline solution to 4.0.

Sedation and Anesthesia

For computed tomography (CT) examinations and intraarterial infusion, each rabbit was anesthetized with ketamine hydrochloride (44 mg/kg; Daiichi-Sankyo, Tokyo, Japan) administered intramuscularly. Subsequent intravenous access was gained via a marginal ear vein and 0.1–0.2 mL (2.5–5 mg) of sodium pentobarbital (maximum dose of 50 mg/kg; Somnopenthyl; Kyoritsu Seiyaku, Tokyo, Japan) was administered periodically to maintain anesthesia.

Intraarterial Infusion Procedure

All infusion procedures were performed by interventional radiologists in the operating room equipped with a C-arm device (SIREMOBIL Compact L; Siemens Medical Solutions, Erlangen, Germany) in our animal laboratory. After sedation and anesthesia, the right femoral artery was exposed and directly punctured with a 24-gauge needle (SURFLO intravenous catheter; Terumo) and the catheter was retrogradely advanced into the distal external iliac artery. The catheter was then connected to an extension tube to allow stable and easy administration of solution during the procedure. After the catheter tip was placed just above the origin of the feeding artery, digital subtraction angiography was performed by hand injection of 1 mL of contrast medium (Iopamidol 300; Bayer, Tokyo, Japan) at a rate of 0.1 mL/s to confirm distribution of contrast medium to the tumor (**Fig 1**). Fifty milliliters of the CO_2-saturated solution, equal to ±20%–25% of intravascular volume, was administered through the catheter for 10 minutes in the CO_2 group, and the same amount of saline solution was administered in the control group. To ensure distribution of the solution through the tumor, we

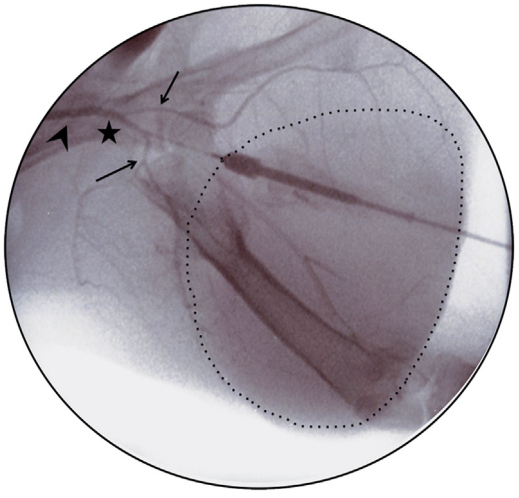

Figure 1. Digital subtraction angiography of the right iliofemoral artery through an intravenous catheter placed in the distal external iliac artery (arrowhead). Note the tip of catheter (star) near the feeding arteries (arrows), which are branches of the distal external iliac and proximal femoral arteries. Contrast agent pooling (ie, tumor staining; dotted line) indicates the tumor lesion. Note that both feeding arteries have many branches around the tumor. (Available in color online at *www.jvir.org*.)