SCIENTIFIC PAPER

Prevention of Vessel Desiccation and Maintenance of Normal Morphology During Endovascular Harvesting Using Humidified Warmed Gas

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ABSTRACT

Background and Objectives: Endoscopic vessel harvesting (EVH) traditionally uses carbon dioxide (CO_2) gas for insufflation. The CO_2 based on government regulations is bone dry and room temperature. All previous EVH studies use this type of unconditioned gas. It is hypothesized that by changing the quality of CO_2 gas differences may occur that are attributable to dry gas versus wet gas exposure.

Methods: A comparison of the effect(s) of traditional dry CO_2 gas compared to humidified exposure was done using a porcine model and evaluated in a double-blind randomized controlled fashion.

Results: Vessels exposed to traditional dry cold gas had morphologic and structural changes noted on histologic evaluation. This included desiccation changes of the tunica adventitia desiccation and tunica media collagen and elastin. Vessels exposed to dry gas showed 10% to 12% contraction and constriction with tortuous changes to the intima and endothelial lining that were progressive with increasing volumes of gas exposure. No desiccation or morphologic changes were seen with humidified warmed gas produced using the VesselGuardian.

Conclusions: Traditional dry cold CO_2 caused vascular tissue damage extending from the adventitia to intima, changing the vessel in morphologic and structural configuration. With the VesselGuardian humidified warmed, gas maintained vessel morphology and integrity by preventing desiccation. Changing the quality of CO_2 from dry and cold to wet and warm may offer clinical utility for a better quality conduit for coronary artery bypass graft procedures.

Key Words: Endoscopic vessel harvesting, Saphenous vein harvesting, Carbon dioxide, Adventitia, Collagen, Desiccation, Coronary artery bypass graft.

INTRODUCTION

A primary cause of vascular graft failure in coronary artery by-pass surgery is mechanical damage to the graft during harvesting including removal of surrounding adventitial tissue.[1] This has led to various techniques to preserve the integrity of endoscopic vessel harvesting (EVH) vessels. Although it is known that prevention of intimal damage during coronary artery bypass grafting is important, little attention has been placed on the prevailing conditions on the outside surface of the vessels harvested, ie, adventitia and vessel wall through to the intima during EVH. The carbon dioxide (CO_2) used for endoscopic vessel harvesting must be considered regarding any tissue effects it may cause. It is well established in laparoscopy that improvements in clinical outcome result when the quality of gas is changed from the harsh dry condition mandated by the Food and Drug Administration[2] to one that is more physiologically appropriate for humidity and temperature. The traditional CO_2 gas used for endovascular harvesting (EVH) is <200 parts per million of water vapor or <0.02% relative humidity making it 100 times drier than the 2% Sahara desert and room temperature or 20°C. The normal prevailing conditions for blood vessels is body temperature 36°C and surrounded by water-saturated cells bathed in interstitial fluid (95%) to maintain their integrity and proper physiologic function.

It is hypothesized that there would be a tissue drying effect to the adventitia and surrounding structures of the saphenous vein when the currently dry cold CO_2 is used during EVH and that this would be reduced or eliminated using humidified warmed gas.

Medical devices used supplied by the manufacturer, LEXION Medical, St. Paul, MN.

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MATERIALS AND METHODS

A protocol was submitted and approved by the Nicholson Center for Surgical Advancement, Florida Hospital Celebration Health, Celebration, Florida. Four veins were harvested from two[2] 31-kg pigs and exposed to 2 different qualities of CO_2. Group 1 was exposed to the currently used CO_2 gas without modification, and group 2 was exposed to humidified warmed CO_2 gas using the *Vessel*-Guardian device. The *Vessel*Guardian is cleared by the FDA and approved for EVH and is indicated for conditioning CO_2 gas for EVH. Vessel harvesting was done by the same experienced person for all veins in both groups. A baseline biopsy of each vessel was taken at time zero with no gas exposure. After harvesting and prior to placement into a holding chamber, the vessels were flushed with heparinized lactated Ringer's solution (1 unit per milliliter (ml) solution) at $<$100mm Hg pressure. Each vessel was placed in a chamber for exposure to either quality of gas, as it would be during endoscopic harvesting. Gas flow rate was 2 liters per minute and 12mm Hg pressure. Vessel exposure to either gas condition was for increasing volume increments up to 60 liters. Vessel biopsies were taken at 0, 6, 12, 30, 45, and 60-liter exposures. All biopsies were given randomized numbers and logged with information related to type and volume of gas exposure. Each biopsy was placed in 10% formalin for histologic evaluation. Tissue specimens were stained by using hematoxylin eosin and Masson's trichrome staining, according to standard laboratory protocols and evaluated by 2 different pathologists using light microscopy. The biopsy sequence was blinded to physicians evaluating the histologic specimens. A reproducible grading system was devised for this study scoring uniformity, continuity and integrity of the intima, elastic membrane, medial smooth muscle, collagen, connective tissue, and adventitial tissue.[3] The grading system estimated percentage disruption of each histologic structure with the scale being 0 (no disruption), 1 ($<$10%), 2 (10% to 25%), 3 (25% to 50%) and 4 ($>$50%).

RESULTS

Vessel biopsies at time zero had normal staining, morphology, architecture, structure, and integrity. The vessels exposed to humidified warmed gas had no peri-vascular changes, did not have adventitial drying or desiccation, had no constriction changes, and had no change in intimal integrity or morphology. Vessels in group 1 exposed to dry cold gas had peri-vascular changes, adventitial drying and desiccation effects, circumferential radial constriction, and progressive convolution of the intima. Vasa vasorum were patent in group 2 and collapsed or unidentifiable in group 1. Compaction and spacing of structural components of the media layers are seen to progressively narrow in Group 2 and remain normally spaced in group 1 as in the control biopsies. Different magnifications are shown because of size changes throughout the length of the vessel. The histology of veins harvested using conventional dry gas compared to humidified warmed gas is seen in **Figures 1 and 2**.

With increasing volume of dry gas exposure, there was progressive disruption and desiccation of the adventitia starting at 6-liter exposure. No adventitial or connective tissue disruption was seen in the humidified warmed group throughout the total 60-liter exposure. There was radial contraction of the vessels in the conventional CO_2 group as the volume of dry gas exposure increased causing convolution, loss of normal histology, abnormal tortuosity, and piling up of the intima lining. Contraction was measured as 10% to 12% in the dry gas group, and there was no loss of original conduit size in the humidified group. Elastic membrane disruption was seen in the dry CO_2 group after 12 liters of exposure. No elastic membrane disruption was noted at any volume of the humidified CO_2 group. The effect of CO_2 quality on vein histology is summarized in **Table 1**.

DISCUSSION

EVH done for coronary artery bypass grafting (CABG) reduces wound infection and postoperative pain, shortens hospital length of stay, and improves patient satisfaction. The real concern is how can EVH improve conduit quality and outcome? Cells in the adventitia serve as an energy store and provide dynamic structural and microvascular support (vasa vasorum) as well as being a source of fibroblasts, endothelial cells, mastocytes, pericytes, reserve stem and progenitor cells, and first responder macrophages and T cells.[4] Adventitial inflammation effects intimal repair as an inward progression. Because these components are sources of nitric oxide, production influencing intimal repair external vascular damage and cell migration affects the potential for intimal hyperplasia formation.[5,6]

Adventitia has been regarded as having a minor role in plaque formation, but it is now realized that any adventitial inflammatory reaction has the potential to initiate atherosclerosis by cytokine production and induced microvascular changes.[7,8] This is described as the "outside in" process of atherogenesis that can be applied to EVH.[9]

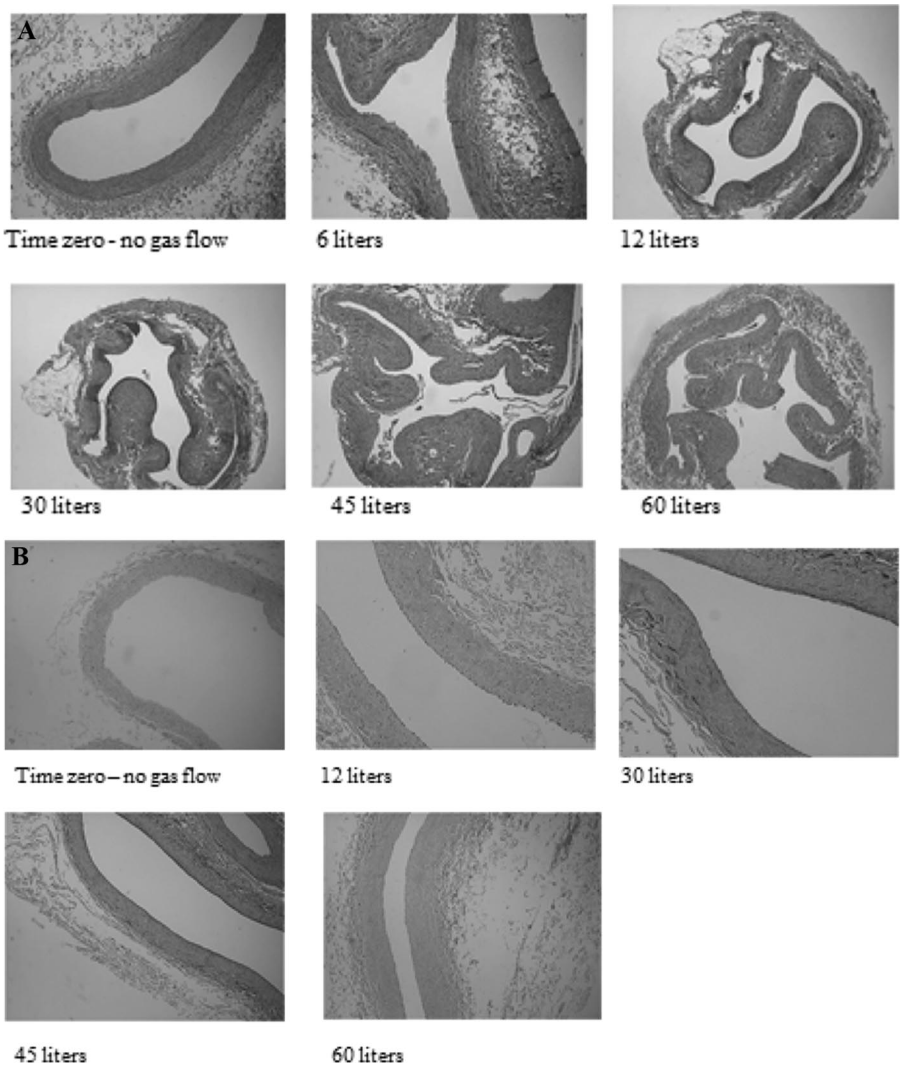

Figure 1. Conventional dry cold CO_2 gas exposure compared to humidified warmed *Vessel*Guardian® gas up to 60 liters volume. (A) Conventional CO2 gas exposure to a harvested vein. Morphologic changes (radial contraction and constriction) of a vein due to dessication caused by dry CO2 exposure with increasing volume of gas exposure. (B) Humidified CO2 gas exposure using the *Vessel*Guardian to harvest a vein. No contraction, constriction or tissue dessication. Vessel structure morphology and architecture remain normal throughout humidified warmed gas exposure.

Therefore, adventitial damage during EVH should be kept to a minimum. A recent report claims that "endoscopic vein-graft harvesting is independently associated with vein-graft failure."[10] This has been refuted since "it is not the EVH technique per se that causes conduit damage and eventual graft failures[11] but other pertinent factors may contribute to the problem."[12]

The only type of gas used in these and all previous studies to date has been unconditioned dry CO_2 not normalized for prevailing physiologic humidity and temperature conditions. Therefore, it could be argued that there has never been an appropriate control, because there is no study until now comparing conditioned wet and warmed CO_2 to the conventional dry and cold CO_2. An explanation for previous abnormal EVH findings including morphologic, structural, and chemical changes and decreased patency could be explained by damage induced due to the gas quality used (virtually no water content and low temperature). It is known in laparoscopy that the quality (moisture content and temperature) of insufflation CO_2 matters. Traditional unconditioned laparoscopic gas causes evapo-

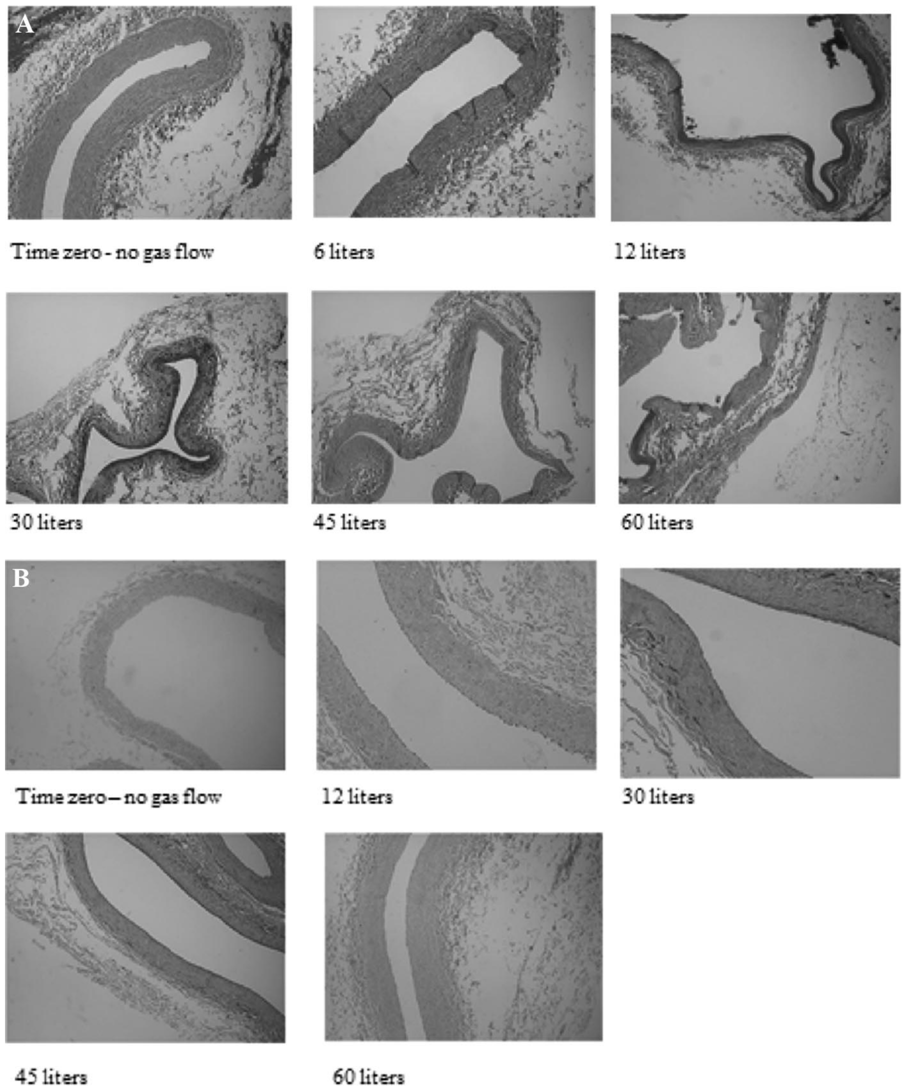

Figure 2. Conventional dry cold CO_2 gas exposure compared to humidified warmed *Vessel*Guardian® gas up to 60 liters volume. (A) Conventional CO2 gas exposure to a harvested vein. Morphologic changes (radial contraction and constriction) of a vein due to dessication caused by dry CO2 exposure with increasing volume of gas exposure. (B) Humidified CO2 gas exposure using the *Vessel*Guardian to harvest a vein. No contraction, constriction or tissue dessication. Vessel structure morphology and architecture remain normal throughout humidified warmed gas exposure.

rative changes resulting in hypothermia, tissue desiccation, and initiation of an acute inflammatory process, traumatized tissue, increased adhesion formation, and negatively effects clinical outcomes. Changing the gas from dry to wet during laparoscopy preserves peritoneal integrity.[13,14] This study's findings regarding changing the moisture content of the CO_2 gas offers similar benefits for endoscopic harvested vessels by preventing desiccation effects, morphologic changes, and loss of structural integrity that could lead to compromised vessel quality, patency, and reduced longevity.

Open vein graft harvesting is associated with risks of serious complications and discomfort. Endoscopic vein harvesting based on randomized and nonrandomized trials has been endorsed as the standard of care.[15-26] Graft occlusion rate is a significant determinant for long-term benefits of either technique with clinical outcomes being influenced by harvesting techniques.[25] The use of ex-

Table 1.
Graded Score Represents the Percent Disruption Scored According to the Scale: 0 (no Disruption), 1 (<10%), 2 (10%–25%), 3 (25%–50%), and 4 (>50%)

Histologic Characteristic	Group 1 Conventional Dry, Cold CO_2	Group 2 *Vessel*Guardian® Humidified Warmed CO_2	Immediate Harvest Control
Intimal endothelial continuity	1	1	1
Elastic lamina continuity		1	1
Medial connective tissue uniformity	2	1	1
Medical smooth muscle continuity	3	1	1
Adventitial connective tissue uniformity	4	1	1
Tissue morphology and architecture	4	1	1

tremely dry CO_2 gas has been an experiment resulting in observations of what happens under abnormal conditions, since there has never been a control gas condition mimicking normal vascular conditions for EVH until this evaluation. These findings show that exposing vessels to unconditioned CO_2 causes morphologic and detrimental changes to occur, which do not occur with humidified gas. The beneficial clinical findings of minimized hypothermia, elimination of tissue desiccation, reduced acute inflammatory reaction and decreased postoperative pain seen in laparoscopy when humidified gas is used should be seen as beneficial effects for vascular structures when moist warm gas is used for endovascular vein harvesting.[27-35]

During vessel harvesting, dry gas exposure to the external surface of the vessel wall and surrounding tissue structures from the outside of the vessel causes shrinkage, resulting in longitudinal contraction and radial constriction. There is a critical point of water loss due to evaporative desiccation of connective tissue surrounding collagen, smooth muscle, and the elastic membrane that leads to irreversible shortening and structural changes of cellular proteins, resulting in reduction in tissue compliance and elasticity. This is seen as an alteration in morphologic appearance and structure of the normal smooth contour of the vessel inner surface to one that is undulating and tortuous. Damage to the adventitia and medial layers of the vessel can contribute to ischemia and result in fibrous proliferation and vascular remodeling. Various patterns of changes are seen along the length of the harvested conduit due to intrinsic tissue variability and different amounts of cumulative effects resulting from direct and indirect dry gas exposure. Tissue drying increases resistance to electric current and can change conditions and requirements for hemostasis and vessel coagulation by increasing undesirable lateral thermal spread.

Coronary artery bypass conduits that have restricted limits of compliance change blood flow characteristics causing increased turbulence. Flow characteristics and dynamics can be altered due to structural changes altering normal morphology of the intima without disturbing endothelial cell chemical function. Increased conduit stiffness of a graft can increase vascular tension and pulse pressure, compromising long-term conduit function and patency. Preventing irreversible changes to vessel wall cellular components, structures, and surrounding surfaces by preventing desiccation is accomplished when humidified warmed CO_2 harvesting is used. The observations made during this evaluation at least partially explain recent negative claims[10] showing an increased rate of graft failure following EVH, because vessel damage was probably and most likely induced by the extreme dry gas conditions.

CONCLUSIONS

Vessel injury occurs due to the very dry condition of the CO_2 used during endovascular harvesting. Humidifying and warming the gas with the *Vessel*Guardian reduces desiccation and vessel damage during endoscopic harvesting and maintains normal histologic and morphologic vascular structure. Further studies are in progress to determine biomechanical and biochemical changes induced by the dry gas endovascular harvesting techniques. Long-term consequences and graft patency may be significantly altered by dry gas conditions. Alternatively, graft conduits are kept in as close to their normal in situ condition by using conditioned humidified warmed gas during EVH. To reduce vessel desiccation from the outside in toward the tunica intima and improve vessel viability and pliability, humidifying the gas during endovascular harvesting is recommended to maintain normal structural morphology and integrity.

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