Carbon Dioxide Contrast Agent for CT Arteriography: Results in a Porcine Model

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PURPOSE: To test the feasibility of carbon dioxide (CO₂)-enhanced computed tomographic (CT)–arteriography in an animal model.

MATERIALS AND METHODS: Five domestic swine underwent digital subtraction angiography (DSA), conventional CT angiography with iodinated contrast material, and CO₂-enhanced CT arteriography. For each CO₂-enhanced DSA image series, 100 mL of pressurized CO₂ was injected at 1.3 bar. CT imaging was performed according to a standardized scan protocol (2 × 32 × 0.6 mm; 120 kV, 210 mAs eff, 330 msec gantry rotation time). Iodinated contrast material was administered intravenously according to a biphasic injection protocol. For CO₂-enhanced CT arteriography, CO₂ was administered intraarterially via a catheter placed in the juxtarenal aorta. An injection pressure of 0.65 bar (volume flow rate, 7.5 mL/sec) was applied. Images were assessed visually by two observers on a four-point grading scale. Absolute intraarterial attenuation values were measured.

RESULTS: Image quality was rated to be the best for standard DSA. CO₂-enhanced DSA was rated slightly superior to CO₂-enhanced CT arteriography. No examination was considered to be nondiagnostic. The average multislice spiral CT (MSCT) scan duration was 7.9 sec ± 0.6. The average amount of gas required for CO₂-enhanced CT arteriography was 104 mL ± 4, compared with 400 mL for CO₂-enhanced DSA. Absolute attenuation values were significantly higher with CO₂-enhanced CT arteriography (aorta, −928 HU ± 39) than with standard CT angiography (490 HU ± 40; P < .0001).

CONCLUSIONS: CO₂-enhanced CT arteriography is feasible. In a porcine model, this technique is capable of depicting the aortoperipheral vessels down to the lower limb. These results warrant further studies of the diagnostic value of CO₂-enhanced MSCT arteriography for the detection of arterial pathologic processes.


Abbreviations: DSA = digital subtraction angiography, MSCT = multislice spiral computed tomography
graphic imaging techniques is the need to administer a contrast agent. Despite the routine use of low-osmolar nonionic contrast material and dedicated premedication in selected patients, iodinated contrast material continues to be associated with nephrotoxicity and allergic reactions. Although iodinated contrast material may be used in patients undergoing hemodialysis, whose renal function is not preserved, it should be avoided in patients with compromised but residual renal function. Recently, the use of MR contrast agents has resulted in severe side effects, particularly in patients with severely compromised renal function in whom nephrogenic systemic fibrosis has been observed (7,8). Therefore, alternative imaging techniques are needed for this particular group of patients.

In patients with contraindications to iodinated contrast material, CO₂ is a well established intraarterial contrast agent for catheter angiography. CO₂-enhanced angiography has also been proven to be an effective alternative to invasive venography (9). Sporadic publications have reported the use of CO₂ in CT for imaging the urinary bladder (10,11), uterine cavum (12), and pulmonary arteries (13). However, to our knowledge, its use for CT arteriography has not been reported to date. The aim of this study was to test the feasibility of invasive CO₂-enhanced CT arteriography in an animal model.

MATERIALS AND METHODS

Animal Preparation

Five domestic swine (mean weight, 52 kg ± 4) were included in this study after approval from the state animal care committee. All examinations were performed under general anesthesia. After intramuscular premedication with 0.5 mL atropine 1% (WDT, Garbsen, Germany), 2 mg/kg azaperone (Janssen-Cilag, Neuss, Germany) and 5 mg/kg body weight ketamine (Ketamin 10%; Ceva, Düsseldorf, Germany), anesthesia was initiated by intravenous injection of 5 mg/kg body weight pentobarbital (Merial, Hallbergmoos, Germany). Animals were orotracheally intubated and mechanically ventilated throughout the entire procedure (Sulla 808; Draeger, Lübeck, Germany). Anesthesia was maintained with isoflurane (Abbott, Baar, Switzerland) administered to achieve an end-tidal concentration of 1.0% and continuous infusion of fentanyl (0.03 mg/kg⁻¹h⁻¹; Janssen-Cilag).

CO₂-enhanced Angiography

Animals were examined in the supine position (Integris V3000; Philips, Hamburg, Germany). A 6-F introducer sheath (Terumo Europe, Leuven, Belgium) was advanced into the right iliac artery and a 4-F angiographic flush catheter with a radiopaque tip (Omni-Flush; Angiodynamics, Queensbury, New York) was placed in the abdominal aorta directly above the level of the renal arteries.

First, CO₂-enhanced digital subtraction angiography (DSA) was performed. Pure medical-grade CO₂ was obtained from a pressurized metal storage container (Linde, Pullach, Germany) via a pressure valve (FMD650-03; DruVa, Eppelheim, Germany). The storage container was connected to a dedicated CO₂ delivery system (CO₂ Angioset; Optimet, Ettlingen, Germany), consisting of a 100-mL syringe, a stopcock, and two flexible tubes for the gas supply (14). The plunger of the syringe was fixed at a 100-mL injection volume. With the pressure valve of the metal container set to 1.3 bar, the delivery system was filled with pressurized CO₂. To flush any remaining air from the CO₂ delivery system, the system was filled with CO₂ and emptied into room air four times as recommended by the vendor. Thereafter, the CO₂ delivery system was connected to the saline solution–flushed pigtail catheter. Directly before image acquisition, 20 mg of n-butyl scopolamine (Buscopan; Boehringer Ingelheim) nonenhanced CT images were acquired as a baseline for CT subtraction angiography. Immediately thereafter, CO₂-enhanced CT images were acquired. To establish optimal conditions for the application of CO₂ as an intraarterial CT contrast agent, the first two pigs were scanned repeatedly with different CO₂ injection techniques, various volume flow rates and volumes of gas, and different scan delays. First, the optimal application technique had to be derived. One approach was to apply the gas via a vascular access cannula with a reflux technique at 1.3 bar gas pressure, as has been described for conventional CO₂ angiography (14). This approach was compared with gas insufflations via a 4-F pigtail catheter placed above the renal arteries according to the same injection parameters as for CO₂-enhanced DSA. Gas was applied via the previously described CO₂ delivery system injecting 100, 200, 300 and 400 mL of gas by repeatedly opening and closing the stopcock. In addition, the catheter was directly connected to the pressure valve of the CO₂ storage container with a 3-mm flexible tube (REF 4097262; B. Braun, Melsungen, Germany). Various pressure settings (0.5, 0.6, 0.65, 0.7, 0.8, 1, and 1.3 bar), resulting in different volume flow rates (6.6, 7.2, 7.5, 7.8, 8.3, 9.5, and 10.6 mL/sec, respectively), were tested. We also evaluated different scan delays (0, 2, 4, and 6 sec). The injected volume of gas was calculated by multiplying the vol-
ume flow rate by the sum of scan time and scan delay.

An interim analysis was performed after examination of the first two animals to determine the optimal protocol for CO₂ insufflations. Catheter-based CO₂ insufflation with a volume flow of 7.5 mL/sec (0.65 bar) and a scan delay of 6 seconds was considered to be the best method and was used for further data acquisition. An additional three animals were examined with these parameters for CO₂ application. The CT scan parameters remained unchanged. For data visualization, CO₂-enhanced images were subtracted from nonenhanced images and thin-slab maximum-intensity projections with a slice thickness of 25 mm and an increment of 15 mm were computed. Axial source images and postprocessed images were analyzed visually.

After completion of the CO₂-enhanced CT arteriography, iodine contrast medium—enhanced CT angiography was performed during end-expiratory breath-hold. The same examination and reconstruction parameters as for CO₂-enhanced CT arteriography were used. Iodinated contrast material was administered via an 18-gauge access in a left ear vein. For contrast enhancement, a biphasic injection protocol starting with injection of 30 mL of nonionic contrast material (Ultravist 300; Bayer-Schering, Berlin, Germany) at a flow rate of 6.5 mL/sec followed by 40 mL at a flow rate of 5.5 mL/sec was used. Contrast medium injection was followed by a 50-mL saline solution chaser bolus injected at a flow rate of 5.5 mL/sec. The scan delay was determined according to the bolus tracking technique. For this purpose, a delay of 4 seconds was chosen after a threshold of 180 HU was reached in the juxta-renal aorta.

Conventional Catheter Angiography

Finally, the animals were transferred to the angiography room again, and standard aorto-peripheral DSA from the renal arteries to the hind legs was performed. Angiograms were obtained during end-expiratory breath-hold at 3 frames per second in the abdomen and pelvis and 2 frames per second in the periphery. For each image series, 30 mL of nonionic iodinated contrast medium (Ultravist 300; Bayer-Schering) was injected at a flow rate of 14 mL/sec.

Data Analysis

For all imaging techniques, images from the renal arteries to the lower limbs were evaluated section by section by a radiologist and a vascular surgeon in consensus with a four-point grading scale as follows: 1, complete contrast agent filling with clear delineation of small vessels throughout the entire data set; 2, complete contrast agent filling and clear anatomic detail, but moderately reduced contrast and/or reduced anatomic detail; 3, circumscribed filling defects and/or reduced contrast and/or reduced anatomic detail with still-unaffected diagnostic image quality; 4, nondiagnostic because of multiple or large filling defects and/or markedly reduced contrast with global deterioration in image quality.

Results of the sectional analysis were summarized at four different levels (abdominal aorta, iliac arteries, femoral arteries, and lower-extremity arteries). The worst sectional score was recorded for the entire level. Using the axial source images of the CT angiography with CO₂ and iodinated contrast material, the mean attenuation values and SDs (in HU) were measured from regions of interest in the ascending aorta, iliac, and femoral arteries. At each anatomic site, measurements were obtained from four subsequent slices.

Continuous variables are given as means ± SD. To find differences in the attenuation values for the different anatomic regions, absolute attenuation values were compared with repeated-measures analysis of variance with post-hoc t tests. A P value less than .05 was considered significant. Because this was an exploratory study, an α-adjustment was not performed.

RESULTS

MSCT and conventional CO₂ angiography were successfully completed in all animals. The first pig died after standard MSCT angiography as a result of anesthesia-induced malignant hyperthermia. In four animals, a distinct blush of the skin of the lower extremities was observed. Mean scan length in MSCT angiography was 68 cm ± 5, resulting in an average scan duration of 7.9 sec ± 0.6. The volume CT dose index was 8.64 mGy. The effective radiation dose was not calculated because this was an animal study. The interval between CO₂-enhanced and iodinated contrast material–enhanced MSCT angiography was 6 min ± 2.

Interim Analysis

For interim analysis, the image data from the first two animals were visually assessed by both readers. Because this was an exploratory data analysis, no quantitative measures were obtained at this point. At pressure settings (volume flow rates) of 0.5 bar (6.6 mL/sec) and 0.6 bar (7.2 mL/sec), partial or insufficient gas filling of the arteries was observed. With higher gas pressures (0.65, 0.7, 0.8, 1, and 1.3 bar) and volume flow rates (7.5, 7.8, 8.3, 9.3, and 10.6 mL/sec, respectively), homogeneous filling of the abdominal and lower-limb arteries was achieved, but with gas pressures (flow rates) greater than 0.7 bar (7.8 mL/sec), the animals showed spontaneous movements of the lower limbs, a sign of pain. Vascular filling also improved with increasing scan delay, and a scan delay of 6 seconds provided the best results.

Data Analysis

Quantitative data analysis was performed on image data from all animals with the use of CO₂-enhanced CT arteriography data acquired at an injection pressure of 0.65 bar with a volume flow rate of 7.5 mL/sec and a scan delay of 6 seconds. With a mean scan duration of 7.9 sec ± 0.6, an average of 104 mL ± 4 of gas was insufflated for CO₂-enhanced CT arteriography.

Image quality was rated to be the best for standard angiography. No examination was rated as nondiagnostic. On average, CO₂-enhanced DSA was rated slightly better than CO₂-enhanced CT arteriography (Fig 1). The score for visually assessed image quality decreased as signal attenuation values decreased. In addition, the results showed a slightly higher variability in image quality for CO₂-enhanced CT arteriography (Table). In two animals, circumscribed filling defects were ob-
served in peripheral vessels (Fig 2). Moreover, in CO₂-enhanced CT arteriography, all animals showed an inhomogeneous gas distribution with incomplete filling of the vessel at the catheter tip (Fig 3). The same effect may be seen on standard DSA. The latter did not affect visual grading, as this was above the renal arteries and therefore outside the target region. In one animal, the introducer sheath caused an obstruction of the right iliac...
artery that was correctly diagnosed on conventional CO2 DSA, as well as with both MSCT techniques. At final standard angiography, complete occlusion of the iliac artery was observed (Fig 4), presumably as a result of subsequent thrombosis.

Quantitative analysis showed that CO2-enhanced CT arteriography provided significantly higher absolute attenuation values in all vascular regions compared with standard CT angiography ($P < .0001$). However, absolute attenuation values decreased in the periphery (Table 1).

**DISCUSSION**

During the past decade, significant advances in ultrasound (US), CT, and MR imaging have enabled these techniques to supplement, and increasingly replace, catheter angiography. In contrast to catheter angiography, all these techniques provide three-dimensional information, which is considered beneficial in the assessment of vessel morphology and lesion composition. Although US is commonly used for screening purposes, MR imaging has become a routine imaging modality for detailed evaluation of the aorto peripheral vasculature. The introduction of multislice CT technology has challenged the position of MR for this application. Its near-ubiquitous availability, short room time, and rapid image acquisition have made MSCT angiography a routine tool that provides high-quality vessel imaging 24 hours a day. Compared with MR imaging, MSCT is considered advantageous with respect to therapeutic confidence and costs for the initial evaluation of peripheral artery disease (15). Moreover, MSCT requires less investment costs for equipment. Nevertheless, there are some drawbacks, particularly the exposure to radiation and contrast material.

Iodinated contrast material continues to be the gold standard for CT angiography, but it is associated with a slight risk of adverse events. The most frequent complication associated with iodinated contrast media is contrast medium–induced nephropathy, particularly in patients with impaired renal function (16). In these patients, MR angiography has been considered the method of choice. However, recent reports of nephrogenic systemic fibrosis—a rare but severe and potentially fatal condition—associated with the administration of gadolinium-based MR contrast media in patients with renal failure have motivated the quest for less problematic examination strategies in patients with impaired renal function. One way might be the use of alternative iodine- and gadolinium-free contrast media.

In catheter angiography, CO2 is a well-established alternative to iodinated contrast media (17–19). Its unique properties result in pulmonary excretion of the gas. After intravascular injection, carbonic anhydrase inside the erythrocytes catalyzes CO2 and water to form carbonic acid, which instantly dissociates into a proton and bicarbonate. The latter diffuses into the plasma, where it dissolves. In general, CO2 is believed to dissolve within 15–20 sec-
onds. During the passage of blood through the lung, the reverse process occurs, resulting in the release of gaseous CO₂ into the alveoli. There are no reports of CO₂-induced nephropathy, and CO₂ is considered an ideal alternative to iodinated contrast material in patients with severely impaired renal function (20). Its safety with respect to renal function has been shown in animal studies, revealing a transient decrease in arterial flow, but no functional changes (21). Combination of the three-dimensional imaging properties of MSCT and replacement of iodinated contrast media by CO₂ appears to be an obvious approach to address the aforementioned limitations of CT angiography. However, when considering CO₂ as a contrast agent for CT angiography, one has to be aware of the invasive nature of the procedure. This has to be weighed against the risk of iodine- or gadolinium-based contrast media.

Whereas iodinated contrast medium mixes with blood, CO₂ displaces blood. This particular property of CO₂ has to be considered with dedicated modes of application. An apparent side effect of intravenous injection of relatively large volumes of CO₂ is the displacement of the blood from the right heart, subsequently resulting in death (22). This particular problem has been shown in animal experiments in which massive amounts of CO₂ were injected intravenously for venography, with subsequent death of the animals. In a more recent animal study of intravenous injection of smaller amounts of CO₂ for imaging of the pulmonary arteries (13), several pigs died during CO₂ injection. Consequently, the intravenous injection of CO₂ has to be considered unsafe unless it is performed with extreme care, and the amount of CO₂ should be kept as small as possible. If correctly performed, intraarterial insufflation of CO₂ is safe in terms of CO₂-related complications, but is associated with the typical side effects of invasive angiography. Although relatively large vascular accesses were used in this study, small catheters (as small as 3 F) may be employed for clinical examinations. This will reduce the number of potential procedure-related complications and will allow CO₂-enhanced MSCT arteriography to become an outpatient procedure (23).

Iodinated contrast material mixes with blood, and intravascular enhancement can be improved by increasing the iodine delivery rate by
increasing the flow rate or using a higher iodine concentration. Conversely, CO₂ displaces the blood and the differential attenuation of the gas-filled vessels compared with the surrounding tissues is shown. After total displacement of blood, additional CO₂ will not improve image quality. Instead it may reflux into unwanted areas or contribute to the potential painful sensations that have been described in CO₂ angiography at particularly high flow rates (24). For the same reason, we sought to find the lowest injection pressure that provided a homogeneous CO₂ filling of the arteries without bolus fragmentation. Because of its buoyancy, CO₂ replaces blood in the nondependent portions of the vessels. Consequently, appropriate positioning of the patient is recommended, with elevation of the examined part of the body by 15°–20°. Because the animals were positioned in a supine position with the legs above the level of the aorta, an additional elevation of the examined extremities was not needed. Nevertheless, we observed an inhomogeneous filling of the aorta in the area of the catheter tip, in which the vessel was only incompletely filled with CO₂. To achieve complete filling of the vessels, we chose a relatively long scan delay that, in theory, permits the complete displacement of blood. Displacement of blood from major vessels may also contribute to the observed blush of the skin. There are several potential expla-
nations for this blush of the skin, including reduced absorption of CO₂, as may be observed in the case of diminished arterial inflow; or partial pressure of the intravascular CO₂, exceeding the capacity of the local venous drainage. Both hypotheses are in accordance with the observation that the blush disappeared spontaneously in all animals shortly after CO₂ injection was ceased.

CO₂ typically forms small gas packets, which may mimic stenosis. In DSA, artifacts from bolus fragmentation are avoided by adding the DSA images. Interestingly, in CO₂-enhanced CT arteriography, this effect was only seen in two animals in a very mild form with only circumscribed incomplete displacement of the blood. These pseudolesions were easily recognized as filling defects from axial source images. The only very limited presence of this effect might be a result of the relatively low temporal resolution of CT compared with standard angiography. In this particular setting, data from a full gantry rotation of 330 msec were used for image reconstruction. During this relatively long period of time, the gas packets continue to move through the vessel. This effect is thought to minimize visible bolus fragmentation. By means of slower gantry rotation times, these filling defects might be reduced further at the expense of increased scan times. Another approach to minimize bolus fragmentation is the use of dedicated catheters, including end-hole catheters as used in this study (25).

With attenuation values less than ~800 HU, there is excellent contrast to the surrounding tissue that typically shows attenuation in the range from ~150 HU in fat to approximately 80 HU in solid organs. In more peripheral vessels, the absolute attenuation decreased. This effect can not be explained by inhomogeneous mixture with blood because CO₂ replaces blood. As CO₂ moves in small packages of gas, the changes in attenuation values may be the result of an interpolation during image reconstruction that partly obscures bolus fragmentation. The higher SD of the CO₂ attenuation measurements is partly related to the presence of the angiographic catheter, which will result in beam-hardening artifacts. Although these artifacts did not affect visual assessment, they resulted in locally increased image noise. The high contrast in CO₂-enhanced imaging is a key for low-dose CO₂-enhanced CT arteriography. Considering the clear contrast between intraarterial CO₂ and the surrounding tissue, increased levels of image noise on low-dose MSCT may be acceptable. This has been shown for low-dose CT of the lung (26,27) and for CT colonography (28,29). Ideally, this approach would permit the acquisition of the image series needed for subtraction angiography at the dose of a single CT angiography procedure with iodinated contrast material.

There are several limitations to the present study. A major technical limitation was our gas delivery system that permitted a variation of only the gas pressure. The CO₂ insufflation technique that was used for CT arteriography with direct connection of the angiographic catheter to the pres-
surized gas container may result in uncontrolled, explosive delivery of gas. The latter not only causes discomfort, but can also result in reflux of gas in unwanted vascular territories. Therefore, the use of dedicated CO₂ delivery systems, as in conventional CO₂-enhanced DSA, is strongly recommended. Dedicated injectors, as are available for CO₂ angiography, may further improve gas application and overcome the problem of potentially explosive gas delivery. Another relevant limitation is the absence of vascular stenosis in the animal model. Because this was a first feasibility study, the use of a vascular pathologic process was omitted. However, the diagnostic accuracy of this technique needs to be evaluated before potential clinical application. Finally, a subtraction technique was used, requiring the data of two image acquisitions and resulting in an increased radiation dose. Moreover, this approach is prone to motion artifacts. However, we chose this approach to provide a similar impression of the vascular tree as that provided by MSCT angiography with iodinated contrast media. In theory, the same effect would be achieved with the use of minimum-intensity projections. However, the latter would be hampered by bowel gas. In the reported setting, the gas in the bowels was subtracted after bowel motion was reduced by the injection of n-butyl scopolamine. Finally, only CO₂-enhanced CT arteriography was evaluated. Considering the results of catheter DSA, CO₂-enhanced CT venography is expected to be feasible, with comparable results (30).

In conclusion, CO₂-enhanced CT arteriography is feasible. It has been demonstrated that this technique is capable of depicting the aortoperipheral vessels down to the lower limb in a porcine model. In patients with severely compromised renal function and contraindications to MR imaging, it may provide an alternative diagnostic approach that provides additional information compared with catheter angiography. However, further technical improvements are needed to avoid visible bolus fragmentation. The reported results warrant further studies of the diagnostic accuracy of CO₂-enhanced CT arteriography for the detection of arterial pathologic processes.

Acknowledgment: The study was supported by a grant of the German Society of Vascular Surgery.

References
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