Short-term Effects of Selective Renal Arterial Carbon Dioxide Administration on the Dog Kidney[1]

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Index terms: Angiography, contrast media, 961.448 • Carbon dioxide • Contrast media, experimental studies, 961.448 • Renal angiography, 961.448

JVIR 1994; 5:149–154

Abbreviations: DSA = digital subtraction angiography, DMSA = dimercaptosuccinate, LM = light microscopy, OIH = iodine-131 iodohippurate, SE = standard error, SEM = scanning electron microscopy, TEM = transmission electron microscopy

PURPOSE: The authors examined the nephrotoxicity of carbon dioxide injected directly into the renal arteries as an arterial contrast agent.
MATERIALS AND METHODS: Fourteen anesthetized dogs received selective renal infusions of CO_2 ranging from a normal dose of 7 cm^3/kg to high doses of 11–54 cm^3/kg. Two dogs received conventional iodinated contrast media. The effects on renal function and histologic appearance were evaluated by means of radionuclide studies (iodine-131 iodohippurate sodium and technetium-99m dimercaptosuccinate) and histopathologic examination (light, transmission, and scanning electron microscopy).
RESULTS: Although there was a mean decrease in renal blood flow of 11.86% (standard error [SE], 7.1) immediately after the injection of CO_2, flow had returned to baseline (0.17%; SE, 5.27) after 24 hours. Although the sample size was small, there was no dose-dependent effect of CO_2 on renal function and histologic appearance. Mild histologic changes and one case of moderate acute tubular necrosis were seen only in cases in which the kidney was positioned vertically rather than laterally.
CONCLUSION: Although formal studies in patients are required, the results of this investigation suggest that CO_2 may be a safe contrast agent and less nephrotoxic than existing contrast agents, providing care is taken to ensure that CO_2 is not trapped in a vertically positioned kidney, as might occur in renal transplant recipients.

ALTHOUGH many new imaging modalities are being developed, angiography continues to be the gold standard for imaging the vascular system. At the University of Florida (Gainesville), we have effectively used carbon dioxide as an arterial contrast agent in over 100 animals and 700 patients (1,2). Since CO_2 is eliminated in a single pass by the lungs, if the injection is distal to the renal arteries, none traverses the renal vessels and therefore no effect on renal function is anticipated. This is a major advantage over traditional contrast agents in patients with a high risk of contrast material–induced nephropathy (ie, those with diabetes or renal insufficiency) (3). However, with the increased recognition of renovascular diseases causing hypertension or decreased renal function, radiologists are frequently presented with the problem of the choice of an imaging agent for aortography or selective renal arteriography in patients at high risk because of impaired renal function. The aim of this study was to examine the nephrotoxicity of CO_2 injected directly into the renal arteries.

Nephrotoxicity is normally associated with functional changes, which include a decrease in renal blood flow, glomerular filtration rate, and structural changes in the tubules or glomeruli. Therefore, we assessed renal blood flow and function in dogs by studying iodine-131 iodohippurate (OIH) (Hippuran; Mallinckrodt, St Louis, Mo) uptake before, immediately after, and 24 hours after intrarenal CO_2 injection and correlated these data with light and electron microscopic changes in the renal tissue collected from animals killed

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1½–3 days after the CO_2 injection (4). Technetium-99m dimercaptosuccinate (DMSA) was used exclusively to detect any focal or segmental parenchymal perfusion defects due to trapping of gas bubbles, clots, or catheter problems. Previous studies evaluating the use of intravenously administered CO_2 had not detected significant renal toxicity (1,2). Moreover, our experience with intraaortic CO_2 in 100 patients revealed no overt toxicity. Therefore, this study was conceived to evaluate the renal toxicity of this imaging agent more thoroughly prior to large-scale clinical trials.

MATERIALS AND METHODS

Sixteen adult canines weighing between 15 and 30 kg were acquired from random sources and were quarantined and conditioned by the University of Florida Health Center Animal Resources Department. These dogs were housed individually in 41 × 74-inch (102.5 × 185-cm) runs with the temperature maintained at 72°F (22.4°C), fed laboratory dog chow (Purina) ad libitum, and cared for in accordance with requirements as stated in the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23, revised 1985). All research with these animals was governed by the principles of the *Guide,* and studies were approved by the University of Florida Committee on the Care and Use of Laboratory Animals.

The 16 dogs in this study were anesthetized with 4% thiamylal sodium and maintained on 1.5% halothane or isoflurane gas. All but the first canine were given 100 U/kg of heparin. Through a femoral artery puncture, a 4-F cobra catheter was advanced into the renal artery under fluoroscopic control. A platinum-tipped, 0.016-inch coronary-type torque wire placed inside the 4-F catheter provided good fluoroscopic visualization of the catheter tip and, with advancement, helped identify the renal artery. If the configuration suggested that the renal artery was indeed entered, an injection of either CO_2 or iodinated contrast media was made with use of digital subtraction angiography (DSA) (ADAC Labs, Milpitas, Calif) 512 × 512 imaging coupled to a 14-inch Philips intensifier at three to five images per second. Fourteen dogs received CO_2 injections. Two dogs received iodinated contrast material: Hypaque-76 (ionic) (diatrizoate sodium meglumine; Sanofi Winthrop, New York, NY) or Omnipaque 200 (nonionic) (iohexol; Sanofi Winthrop). CO_2 was delivered with a dedicated CO_2 injector, which delivers the CO_2 in a controlled manner at a minimal injection rate of 7 cm^3/sec. The delivery system included a constant saline flush, which provided automatic infusion of saline immediately after each CO_2 injection via two one-way valves and a pressure bag. Initially, CO_2 was injected at a rate of 7 cm^3/sec for 3 seconds. DSA images were obtained during all the injections. If contrast material refluxed from the selective site into the aorta and subsequently into the contralateral kidney, these animals were placed in a second group ($n = 4$) in which larger amounts of CO_2 were delivered. Injections were made approximately every 2 minutes, for a total of 12–13 injections. The lateral position (test kidney anterior) was used in the first eight canines injected with CO_2. In the last six canines, the animal was rotated in a prone oblique position in an attempt to place the kidney directly above the injection site.

After the injections were completed, the catheter was removed and the animal was taken to the nuclear medicine laboratory. One day prior to, immediately after, and 24 hours after the CO_2 injection, renal function was evaluated with intravenous injections of 200–300 μCi (7.4–11.1 MBq) of OIH, a renal function imaging agent. The uptake of OIH is directly proportional to effective renal blood flow (3). Imaging was performed for 20 minutes at 1 minute per frame with use of a large-field-of-view gamma camera equipped with a high-energy collimator and interfaced to a standard nuclear medicine computer. Differential uptake was calculated by using the 2nd and 3rd minutes of data after OIH injection. A renogram (time activity curve for each kidney) was derived from computer analysis of the data.

Five to 6 mCi (185–222 MBq) of Tc-99m DMSA, a renal cortical imaging agent, was injected intravenously at baseline and 24 hours after CO_2 infusion. Anterior and posterior 3-minute images were acquired with use of high-resolution collimators and "geometric mean" images to correct for varying attenuation. Differential function was again evaluated, and the scans were analyzed for evidence of global reduction in perfusion or small defects in perfusion (ie, small vessel spasm, thrombosis, infarct, etc).

Thirty-six to 60 hours after CO_2 infusion, the dogs were anesthetized as previously mentioned and prepared for nephrectomy. The renal arteries were exposed by blunt dissection through an abdominal midline incision, which was extended caudally and laterally at the level of the kidneys. The renal arteries were occluded with vascular loops and clamped, and bilateral nephrectomies were performed. The anesthetized dogs were killed with high-dose sodium pentobarbital. Wedge sections of the kidneys were placed in buffered 10% formalin for light microscopy (LM). Wafer-thin sections of the cortex were placed in Trumps medium and prepared for transmission electron microscopy (TEM) according to Croker and Tisher (5). Sections of kidney were also taken and immersed in 2% glutaraldehyde in phosphate-buffered saline or Tyrode buffer for scanning electron microscopy (SEM) according to Nation (6). A core sample of tissue from the renal cortex was placed in Michel medium, frozen, and stored in a deep freezer at −30° to −70°C for direct immunofluorescence study (5). Sections of renal artery proximal to the bifurcation were also taken for LM and SEM.

Findings at Histopathologic Study 24 Hours After CO_2 Injection in Nine Group 1 Dogs

Dog	Effect	Kidney Position
1	Acute tubular necrosis, <1%*	Lateral
2	No visible lesions	Lateral
3	No visible lesions	Lateral
4	Endothelial swelling†	Oblique
5	Acute tubular necrosis, moderate to severe*	Oblique
6	Vacuolization of epithelium, endothelial swelling†	Oblique
7	No visible lesions	Oblique
8	No visible lesions	Oblique
9	No visible lesions	Oblique

* Seen at LM.
† Seen at TEM.

Ten dogs received selective injections of CO_2 at 7–8 cm^3/kg (group 1) with use of the contralateral kidney as the control. Four additional dogs demonstrated reflux into the contralateral kidney; thus, the contralateral kidney was not used as a control. These four animals were designated as a second group (group 2), which received a total volume of 11–13 cm^3/kg (three dogs) and 54 cm^3/kg (one dog) at a much higher injection rate.

Two animals were excluded from comparison group data analysis. The first animal studied did not receive heparin. We suspect that the dog sustained transient renal artery thrombosis, since OIH uptake was absent immediately following CO_2 injection but returned to normal 24 hours later. All other animals received heparin. In the other animal, from the large-dose group, the injection catheter was found in a wedged position. A wedge defect on the DMSA scan obtained at 24 hours confirmed this suspicion.

The mean change in blood flow immediately and 24 hours after injection was calculated for the two CO_2 dose levels (normal vs high), for iodinated contrast media, and for the two positions of the kidney (oblique and lateral) in which CO_2 was injected. The results were tested for significant differences among the three groups with use of the Kruskal-Wallis analysis of variance test.

RESULTS

• Histologic Study

Among group 1 dogs that were positioned laterally (Table, dogs 1–3), one animal demonstrated only minimal epithelial injury in less than 1% of the tubules (7). In this group given a normal CO_2 dose, three dogs with elevated kidneys (prone oblique position) (Table, dogs 4–6) showed minimal changes. In one such dog, LM demonstrated a band of focal tubular necrosis extending from the papillae to the capsule. This kidney was optimally elevated and was the only one in which a definite nephrogram phase was seen with DSA. Although the sections showed significant acute tubular necrosis, the nuclear medicine studies failed to depict ischemia. Endothelial swelling was demonstrated in two kidneys in this group when studied with TEM; in one kidney vacuolization of the tubular epithelium was also seen. These findings are considered evidence of minimal and readily reversible renal injury.

There were no histologic changes in the high-dose group (group 2); all kidneys were injected in the lateral position.

There were no changes detected in the renal artery with LM or SEM in any group. Direct immunofluorescence studies were not considered necessary.

There were no histologic lesions observed in the two dogs in which iodinated contrast material was injected.

• Radionuclide Study

The nine dogs (group 1) that received a normal dose of CO_2 experienced a transient reduction in blood flow of −6.1% (SE, 8.37; confidence interval, −14.5, 2.2), which returned to normal after 24 hours (+2.9%) (SE, 7.54; confidence interval, −1.52, 10.44) (Fig 1). In six of these nine dogs the test kidney was in a raised prone oblique position, and a 1.94% (SE, 11.32) reduction in flow was shown immediately after CO_2 injection. Three of these six (raised prone oblique group) demonstrated a slight delay in peak uptake of OIH and decreased renal clearance immediately after CO_2 infusion. The half-time of clearance of OIH increased from 4 to 8 minutes. This clearance time returned to normal after 24 hours. Histologic change was seen in only one of these three and consisted of focal tubular necrosis.

The mean change in blood flow in the group in which the kidney was laterally positioned was −14.6% (SE, 12.4) immediately after CO_2 and 2.64% (SE, 5.4) 24 hours after. There was no delay in uptake or decreased renal clearance in any of the group 1 and group 2 dogs injected in the lateral position with CO_2. There was no significant difference in blood flow change between the two position groups immediately after CO_2 infusion ($P = .1014$) and 24 hours after ($P = .9607$).

In the high-dose reflux study (group 2), all three dogs experienced transient changes in blood flow and function (−3.18%) (SE, 41.2), which essentially returned to normal after 24 hours (−7.6%) (SE, 13.4). As stated before, there were no histologic changes observed in this group.

The mean change in renal blood flow and function immediately after CO_2 infusion in the 14 dogs that received CO_2 injections was −11.86% (SE, 7.1). If the two experiments in which technical difficulties occurred were excluded, the mean change was

−5.1% (SE, 2.2). The mean residual change in renal blood flow at 24 hours after CO_2 administration was +0.17% (SE, 5.27). If the two technical problem experiments were excluded, the mean change was +0.5% (SE, 2.2).

The mean change in renal blood flow immediately after injection in the two dogs that received Hypaque 76 or Omnipaque 200 was −10.5% (SE, 71.3). The mean change in blood flow at 24 hours was 2.83% (4.9). There were no significant differences in renal uptake or blood flow among the three dose groups (normal, high, iodinated contrast material) immediately after injection (P = .8015) and 24 hours after (P = .2184).

The DMSA study did not reveal any systemic segmental change in function in the three groups at 24 hours after CO_2 injection.

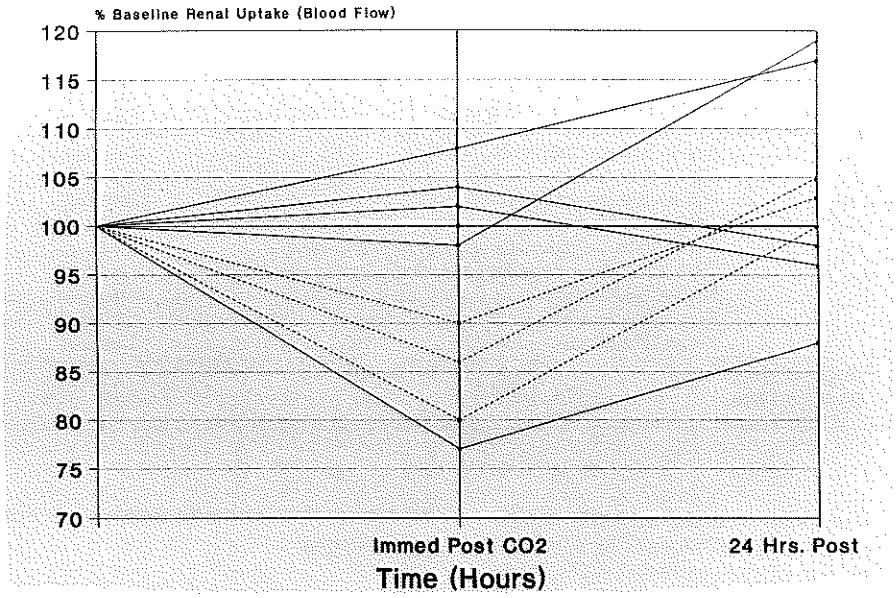

Figure 1. Graph depicts radionuclide OIH change in renal uptake (blood flow) as a percentage of baseline in group 1 dogs that received a normal dose of CO_2 (n = 9). Position of kidneys during injection is designated as lateral (dashed lines) or oblique (solid lines).

• DSA Imaging

Since the dogs' respirations were not suspended, most of the CO_2 DSA images demonstrated motion artifacts. However, usually at least one image with good arterial filling could be obtained if the correct mask could be found. Essentially, all of the kidneys demonstrated uniform filling of all segmental arteries as well as the arcuate. A good cortical phase and nephrogram were seen in only one case, and the renal veins were seen in less than 20% of the dogs. The filling of the kidney was subjectively considered better when the kidney was in the oblique prone position (Fig 2).

DISCUSSION

Although progress in reducing renal nephrotoxicity of angiographic contrast agents has occurred, even the newer nonionic contrast agents can cause renal failure. Indeed, the nonionic agents can accelerate renal failure in patients with significant existing renal disease (8–10). If iodinated contrast material is injected, either intraarterially or intravenously, the kidney is exposed to the agent for a considerable length of time, depending on the ability of the kidney to eliminate the contrast material. However, if CO_2 is administered caudal to the renal arteries, either arterially or intravenously, the kidneys are not exposed to the effects of CO_2, since it is eliminated by the lungs in a single pass.

With the use of CO_2 for standard aortography and selective renal angiography, the peripheral interlobular renal arteries are poorly filled in the supine position. The buoyancy of CO_2 tends to result in reflux of the CO_2 from the renal arteries into the aorta since the kidney is positioned lower than the injection site. In contrast, anteriorly located arteries, such as the celiac, superior mesenteric, and inferior mesenteric arteries, always fill well in the supine position. The first three animals were placed in the lateral position with the test kidney higher or on the same level as the injection site to improve the display of the distal renal arteries. In the last six animals an attempt was made to place the kidney directly above the catheter (semiprone oblique position).

Although there was a mean decrease (11.86%) in the renal blood flow immediately after the injection of CO_2, the flow had returned to normal after 24 hours. Delayed excretion of the OIH occurred only in the group with the vertically oriented kidneys. It is unlikely this represented renal tubular necrosis, since at examination 24 hours later, acute tubular necrosis was detected in only one kidney. These temporary decreases in blood flow are consistent with other canine experiments in which iodinated contrast media was injected into renal arteries or veins (11–13).

It is possible that the delayed OIH excretion represented a physiologic change in renal function associated with an enhanced rate of tubular fluid reabsorption. However, the histologic changes, although mild, were also seen primarily in the cases in which the kidney was vertical. Severe acute tubular necrosis was only seen with the kidney that was elevated, as would be ideal for evaluation of the

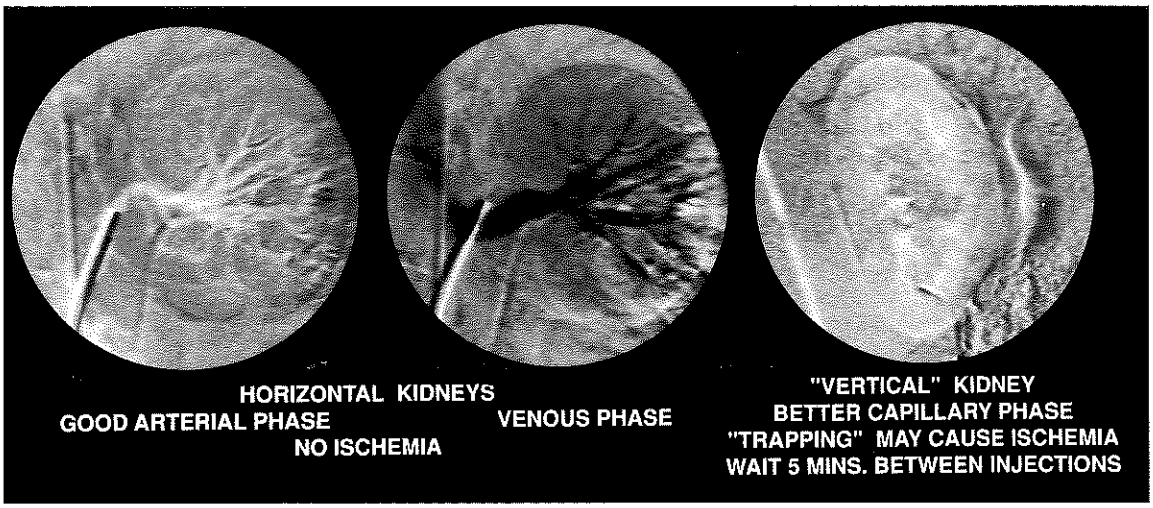

Figure 2. DSA images with injected CO_2 in the lateral or horizontal plane and the vertical or oblique plane.

distal renal vessels. In the kidney, the OIH study demonstrated minimal functional change, again returning to normal within 24 hours. DSA demonstrated an excellent nephrogram, which was not seen in the majority of other dogs. Thus, the results suggest that although a vertically positioned kidney may be ideal for imaging of renal vessels with CO_2, this is associated with an increase in nephrotoxicity and therefore cannot be recommended.

It is possible that the histologic changes were focal and could have been missed in our study, as the histologic samples were obtained at random. We do not know if samples were taken from that part of the kidney that was either higher or lower during the CO_2 injection. The buoyancy of CO_2 causes more exposure at the highest point and least exposure at the lowest point. Regardless, the DMSA study failed to reveal any systemic segmental change in function at 24 hours after CO_2 injection.

The lack of lasting impairment of renal function is consistent with the lack of appreciable changes in renal histologic appearance. All impairment of renal function was transient, and function returned to normal in 24 hours. Only one dog with transient reduction had severe histologic damage. The SEM studies of the endothelium of the arteries should be most informative, since the bifurcation of the segmental arteries was clearly exposed to a very large volume of CO_2 over a period of approximately 1 hour. However, there were no changes observed with SEM in any of the specimens. This contrasts with results of studies by Gospos et al in which significant endothelial damage was demonstrated with SEM and TEM in the rat aorta after administration of both ionic and nonionic contrast material (14,15).

Preliminary ultrasonic CO_2 imaging in two animals not included in this study demonstrated trapping of CO_2 for up to 2 minutes if the kidney was elevated (vertically positioned), whereas the CO_2 bubbles disappeared in less than 30 seconds when the kidney was positioned horizontally at the same level as the catheter. If the CO_2 remained in the majority of the vessels for as long as 2 minutes after injection and the injections were repeated every 2 minutes, the kidney would be exposed to CO_2 continually throughout the procedure. This could cause ischemia. Since we have frequently noted venous opacification after arterial injections in previous studies, we believe that CO_2 does not block capillaries but may be trapped in the apex of any inverted U-shaped vessel. If the organ is higher than the injection site, even if the CO_2 does flow through capillaries, it must be expelled against gravity to return via the veins into the inferior vena cava. It is unlikely that the venous flow is sufficiently rapid to carry the gas against gravity to the lower inferior vena cava.

In the animal in which there was no flow immediately after the CO_2 injection, we initially thought that the renal artery clotted, since the animal did not receive heparin. It is possible, however, that CO_2 was trapped in the kidney after the last injection and was still in the renal tissues during the initial OIH imaging to account for the absence of OIH uptake at that time. This is not very probable since the CO_2 cleared in less than 6 seconds in all injections from the major renal arteries, as evidenced with DSA. Catheter-induced spasm is also a possibility; however, no spasm was appreciated during any of the CO_2 injections.

In this study we used a dedicated injector that delivers CO_2 in a very controlled manner without the possibility of air contamination or high-pressure delivery. The compressibility of CO_2 can lead to an explosive delivery rate. The minimal injection

rate of the dedicated CO_2 injector was 7 cm^3/sec. In four animals, CO_2 refluxed from the renal artery into the aorta, thus exposing the control kidney to CO_2. In these animals, much larger injection rates and volumes were used, attempting to subject the kidneys to even higher doses of CO_2. In actuality, some of the excess CO_2 refluxes into the aorta, thereby exposing the contralateral kidney to more CO_2, but not appreciably increasing the selective volume or rate to the test kidney.

The study has several deficiencies, including (*a*) a catheter was not placed in the control kidney; (*b*) the exact location of the kidney during the injection was difficult to ascertain; and (*c*) the samples for electron microscopy and LM were obtained randomly.

In conclusion, this study demonstrated that large doses of CO_2 injected selectively into the renal artery caused no significant decrease in renal function at 24 hours. The only exception was a 25% decrease in function of the kidney in which a technical problem caused by an infarct occurred. The transient changes in renal blood flow that were observed appeared to correlate with CO_2 trapping in the vertically oriented kidney. In this position, if large volumes of CO_2 are injected very rapidly, injury may occur. This is most important in renal transplants when the kidney is anterior to the injection site but usually is still horizontally positioned. To reduce the possibility of ischemia, we recommend that at least 5 minutes should elapse between CO_2 injections and that the kidney should be rotated between injections.

The absence of a dose-dependent effect of CO_2 on renal function or histologic appearance is particularly encouraging and suggests that, if used appropriately, particularly with regard to positioning, it may have a wide therapeutic margin and could be significantly less nephrotoxic than existing contrast agents. However, the true rate of nephrotoxicity with CO_2 will require properly conducted studies in humans.

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